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Influence of Chemotherapeutics on the Metabolism of Bile Acids in the Intestine of Rats.[1]

Steroids and Bile Acids 17.

By

ARNE NORMAN.

Received 6 September 1954.

It is well known that bile acids are constantly withdrawn from the enterohepatic circulation and excreted via the faeces. A number of old observations have shown that the bile acids present in bile cannot be found in the faeces; it is therefore apparent that the bile acids must have been metabolized during their passage through the intestinal tract. Several investigators have established the transformation of cholic acid by intestinal microorganisms into a number of products (EXNER and HEYROVSKY 1908, LICHT 1924, HUGHES et al. 1942). As to the splitting of the conjugated bile acids it has not been confirmed whether this is due to tissue enzymes or intestinal microorganisms. LICHT (1924) investigated the action of faecal suspensions and of pure strains of bacteria isolated from faeces on the conjugated bile acids and found no splitting of the peptide bonds. FRANKEL (1936), however, has isolated a bacterial strain from human faeces capable of hydrolyzing conjugated bile acids. The action of the tissue enzymes in splitting the conjugated bile acids seems to be of minor importance (cf. GRASSMAN and BASU 1931).

[1] A preliminary report of this work was read at the meeting of the Swedish Biochemical Society in Lund, 3—4 June 1954 (NORMAN 1954).

Recently BERGSTRÖM and NORMAN (1953), using reversed phase partition chromatography for separation of bile acids, have investigated the labelled products in bile and faeces after injection of cholesterol-4-^{14}C. This compound is transformed and excreted in the bile to more than 90 per cent as taurocholic acid. A minor component of the bile acids consists of chenodesoxycholic acid present as taurine conjugate (BERGSTRÖM and SJÖVALL 1954). However, only a minor part of the labelled product in faeces consists of taurocholic acid. Most of the conjugates have been split and the cholic acid further modified.

The present investigation was performed in order to obtain some information regarding the rôle of the microorganisms in the biological splitting of the conjugated bile acids and the further modifications of the bile acid molecules. By suitable chemotherapeutic treatment the faecal aerobes and anaerobes can be reduced to an extremely low level so that one can practically eliminate the action of microorganisms upon the bile acids. In this work a chromatographic separation and identification of the labelled faecal bile acid derivatives was made after the injection of cholic acid-24-^{14}C to rats which, during the experiment, were treated with sulftalyl and terramycin.

Experimental.

Carboxy-labelled cholic acid has been used in these experiments. It was prepared by BERGSTRÖM, ROTTENBERG and VOLTZ (1953) and had a specific activity of about 38,000 c.p.m. per mg when counted in infinitely thin layer on aluminum planchets under a Tracerlab TGC 2 G.M. tube.

Treatment of rats.

Male rats weighing about 200 g were used. 100 mg of terramycin and 250 mg of sulftalyl (phthalylsulfonazolum) (Pharmacia, Sweden) mixed with one ml of water was given by stomach tube twice daily to the rats. Three days after the chemotherapeutic treatment was begun, 3—3.5 mg of ^{14}C-labelled cholic acid was administered intraperitoneally as an aqueous solution of its sodium salt. After the injection of the bile acids, the rats were kept in metabolism cages with free access to white bread and water. Additional vitamins were given once daily together with the antibiotics. The faeces were collected daily into 80 per cent ethanol during a period of four days.

Bacteriological examination.

The faeces of each animal were examined bacteriologically before administration of antibacterial agents, and two days and five days after

initiation of the treatment. Freshly excreted faeces from the rats were suspended in 0.85 % NaCl and serial dilutions were made from the suspension. An estimate of total viable aerobes and anaerobes was made using agar plates incubated at 37° for 24 hours. In both last counts the number of bacteries per unit volume was reduced to approximately a ten thousandth of the one observed in the sample taken before chemotherapy. In none of these rats there was observed any increase in the count on the fifth day due to resistant strains.

Extraction of faeces.

The faeces collected during each 24-hour period were homogenized and refluxed with 80 per cent ethanol for six hours. The cooled solution was filtered and the residue extracted twice with boiling 80 per cent ethanol. It was established that these three extractions were sufficient to isolate all labelled products from the faeces. The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in a small volume of water (30—40 ml), acidified to pH 1 with hydrochloric acid and extracted three times with 2 volumes of butanol. To get a rapid separation of the two phases, the mixture was centrifuged. The combined butanol phases were washed with water until free of hydrochloric acid and then dried with anhydrous Na_2SO_4 and filtered. After evaporation to dryness *in vacuo*, the residue was distributed between equal volumes (50 ml) of 70 per cent ethanol and light petroleum by a three-stage counter-current distribution to remove, among other substances, the fatty acids. The major part of the unsaponifiable material entered the petroleum phase and the rest was separated from the bile acids by partition chromatography as described below.

Fractionation and isolation of labelled bile acids.

The residue from the 70 per cent alcohol phase containing all the labelled products was next subjected to reversed phase partition chromatography as described in earlier papers of this series (Bergström and Sjövall 1951, Sjövall 1953, Norman 1953).

The faecal extract containing the bile acids was first chromatographed with solvent system C to separate all the taurine conjugated bile acids as one group from the glycine conjugates and the free bile acid. For reference to chromatograms with synthetic conjugated bile acids cf. Norman (1953).

Phase system C. 150 ml of methanol, 150 ml of distilled water, 15 ml of iso-octanol, 15 ml of chloroform were mixed in a separating funnel. 4 ml of the lower phase was used per 4.5 g of hydrophobic Supercel.

The size of the column was varied according to the amount of faecal extract. For each 50 mg of faecal solids from a butanol extract, 4.5 g of Supercel and 4 ml of the stationary phase were used. In most cases 13.5 g of Supercel was used for the separation of a 24-hours' faecal extract.

The columns were eluted with 200 ml of moving phase per 4.5 g of Supercel and the effluent collected in test tubes by using an automatic fraction collector. The material remaining in the stationary phase was then eluted with chloroform and its ^{14}C-activity determined. If labelled compounds were found, they were separated with a solvent system for free bile acids described by BERGSTRÖM and SJÖVALL (1951).

The taurine conjugates leave the columns with or just after the front. Of this only an aliquot was used for titration and ^{14}C-determination. It has been found by experiment with synthetic taurocholic acid-24-^{14}C that evaporation of the fractions on the copper planchets resulted in slight hydrolysis. The main part of the eluate containing the "taurine-peak" was therefore evaporated *in vacuo* at 60° and the residue chromatographed with the solvent system D found suitable for the separation of the individual taurocompounds.

Phase system D. 300 ml of water and 100 ml of n-butanol were mixed in a separatory funnel. 4 ml of the upper phase was used per 4.5 g of hydrophobic Supercel.

Taurocholic acid and the more hydrophilic bile acids leave the column with or just after the front. An aliquot of the fractions in the front peak (10—22 ml) was used for titration and ^{14}C-determination and the remainder chromatographed with solvent system E. to separate the taurocholic acid from other compounds.

Phase system E. Moving phase: 510 ml of iso-amyl alcohol and 90 ml of chloroform. Stationary phase: 200 ml of distilled water. 9 g of hydrochloric acid washed, ordinary kieselguhr was used as supporting medium. For the preparation of columns for ordinary partition chromatography see NORMAN (1953).

In order to be able to compare the results obtained in the above chromatographic separation of biological material with those obtained previously with synthetic bile acids (both free and conjugated), the chromatograph patterns have been reduced in scale. The volumes in ml of the moving phase obtained in the present study are hence given in volume corresponding to a column of 4.5 g hydrophobic Supercel. The number in brackets before *ml effluent* indicate how many times larger than a standard (4.5 g Supercel) column the column actually used was, and how many times larger was the actually effluent volume. The amount of moving phase used for eluting a certain band is directly proportional to the size of the column.

Results.

Cholic acid is the main bile acid present in rat bile (BAYERS and BIGGS 1952, BERGSTRÖM 1952, BERGSTRÖM and SJÖVALL 1954. When labelled cholic acid is given intraperitoneally to rats it is excreted in the bile almost totally conjugated with taurine. Only 1—2 % of the labelled cholic acid appeared to be excreted

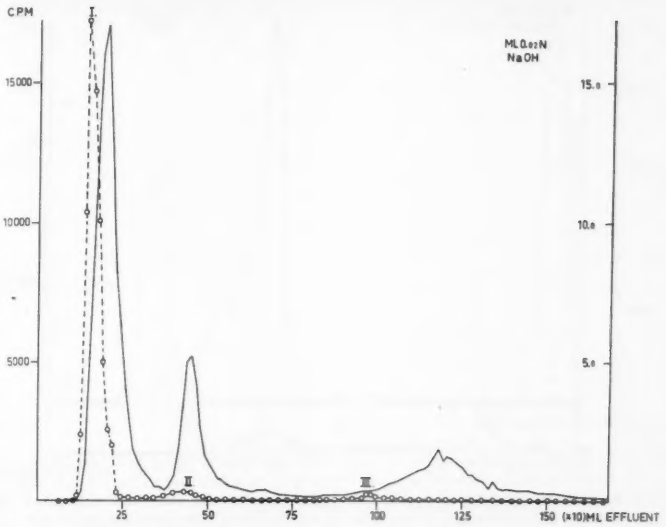

Fig. 1. Chromatography of faecal bile acids excreted during four days after intraperitoneal administration of 3.0 mg cholic acid-24-^{14}C to chemotherapeutic-treated rat. Column: 45 g hydrophobic Supercel. Phase system C, see page 101.

as its glycine conjugate, and none of the labelled cholic acid was found free (NORMAN 1954). After alkaline hydrolysis of the conjugates the only labelled product found was cholic acid (BERGSTRÖM, ROTTENBERG and SJÖVALL 1953). However, only a small part of the bile acids in the bile can be recovered from the faeces in the form in which they are present in the bile. The conjugated acids were found to undergo splitting in the rat and the bile acid molecules were also modified in various ways.

By administration of terramycin and sulftalyl to rats, the total count of faecal microorganisms was considerably reduced so that one can almost consider that the intestinal channel has become sterile. 2.5—3 mg cholic acid-24-^{14}C were given to rats which had been treated previously with chemotherapeutics during three days. This amount roughly corresponds to about ten per cent of the bile acids present in the rat. The bile acids were extracted from faeces and subjected to chromatographic analysis as described above. Figs. 1 and 2 illustrate chromatograms of faecal extracts from two rats. The ^{14}C-labelled bile acids are recovered in three peaks: the

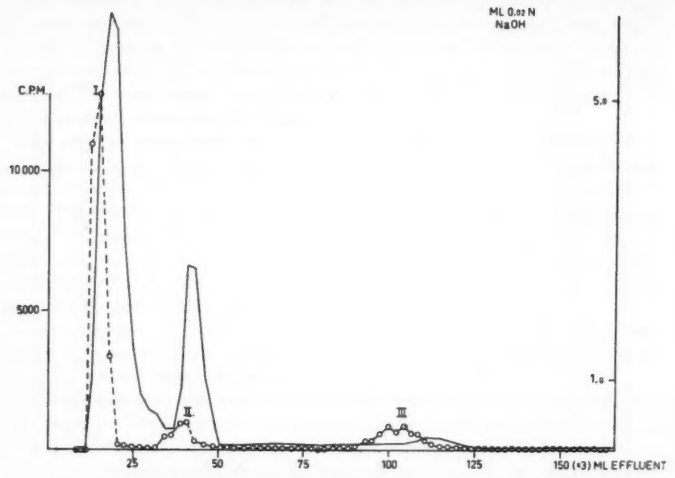

Fig. 2. Chromatography of faecal bile acids excreted on the third day after administration of 3.0 mg cholic acid-24-^{14}C to chemotherapeutic-treated rat. Column: 13.5 g hydrophobic Supercel. Phase system C, see page 101.

main part (95 and 75 per cent) in the front band where the taurine conjugates appear, and the remaining labelled products are eluted at 30—40 ml and 90—105 ml. No ^{14}C activity was found in the stationary phase after it was removed from the column with chloroform. Similiar results were obtained in three more animals. The front band was rechromatographed with solvent system D (fig. 3) and all the activity was found in the front containing the taurocholic acid. No taurocompounds of other bile acids than cholic acid were found in this chromatography. When solvent system E was used in the chromatography, the labelled products were found on elution, together with the unlabelled taurocholic acid added as carrier (fig. 4). The material in the peak was washed off the planchets, hydrolysed, and rerun in solvent system C with cholic acid as carrier. The cholic acid band contained all active material. The material in the band was dissolved in alcohol and diluted with an alcoholic solution of cholic acid and recrystallized as described by BERGSTRÖM (1952). The specific activity remained constant after the first recrystallization.

The labelled compounds present in the two minor bands (II and III figs. 1 and 2) have not yet been identified. They can regu-

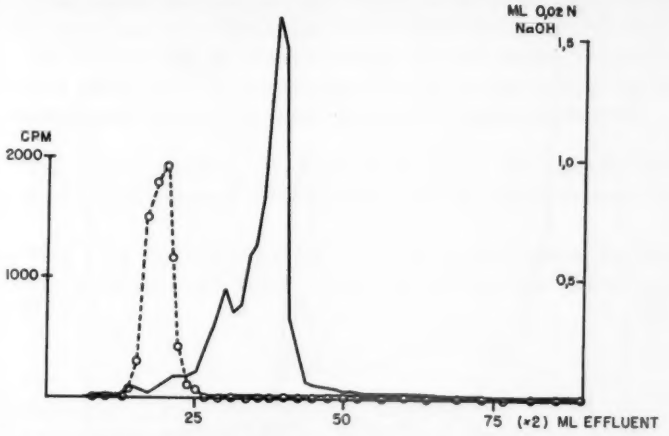

Fig. 3. Chromatography of material from the "taurine conjugate band" (fig. 2) (10—25 ml effluent). Column: 9 g hydrophobic Supercel. Phase system D, see page 102.

larly be separated from all faecal extracts after antibiotic treatment. Their proportion to each other and to the taurine conjugates varies from one animal to another. They were eluted at the same places, respectively, as glycocholic acid at 30—40 ml and free cholic acid at 90—105 ml. After alkaline hydrolysis of the band II, the labelled compound chromatographed with solvent system C

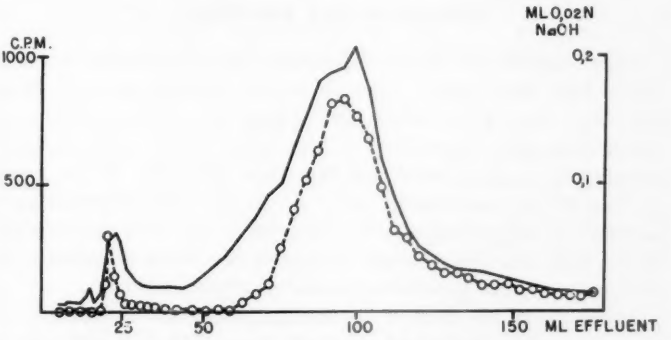

Fig. 4. Chromatography of material from the "taurocholic acid band" (12—25 ml effluent) (fig. 3) with unlabelled taurocholic acid. Column: 9 g Supercel. Phase system E, see page 102.

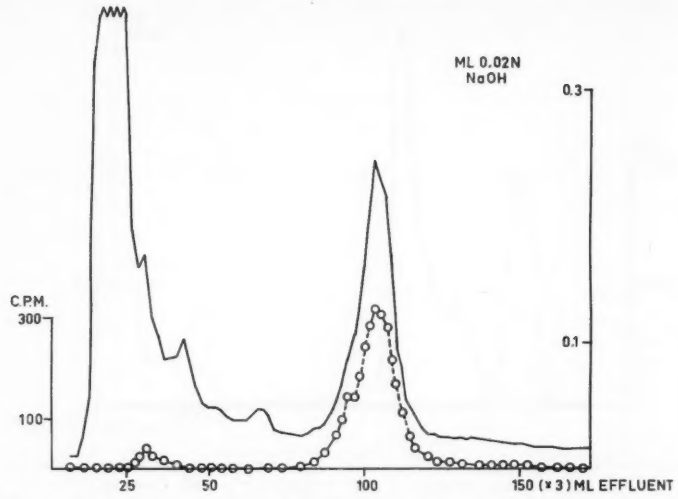

Fig. 5. Band II (fig. 2) (30—40 ml effluent) hydrolysed and rerun with 15 mg unlabelled cholic acid. Column: 13.5 g hydrophobic Supercel. Phase system C, see page 101.

behaved as free cholic acid (fig. 5). Band III was rechromatographed along with free unlabelled cholic acid. All the labelled material moved with the unlabelled cholic acid.

Discussion and Summary.

Cholic acid-24-^{14}C was administered intraperitoneally to rats which had been given terramycin and sulftalyl per os twice daily for three days before and during the whole experiment. Chromatographic separation of the labelled bile acid products excreted in faeces showed that the major part (75—95 per cent) is excreted as taurocholic acid. 2—10 per cent is presumably excreted as glycocholic acid. Likewise in the bile the labelled cholic acid has been found mainly in the form of taurocholic acid, only a minor part being present as glycocholic acid.

About 1—15 per cent of the labelled compound is found at the place where free cholic acid occurred. None of the bile acid metabolites found in normal rat faeces is observed after administration of antibiotics. This indicates that most of the peptide bonds of

the conjugated bile acids are split by the intestinal microorganisms and that these latter also cause modification of the bile acid molecules. Further work is needed to decide whether the small amounts of free bile acid found in these experiments are due to splitting by resistant microorganisms, fungi or intestinal digestive enzymes.

The author wishes to thank Dr. RUNE GRUBB for valuable help with the bacteriological determinations and for helpful discussions.

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Vasoconstrictor Inhibition and Vasodilator Activation — Two Functionally Separate Vasodilator Mechanisms in the Skeletal Muscles.

By

PERCY LINDGREN and BÖRJE UVNÄS.

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The oblongate medulla occupies a key position in the control of the peripheral blood flow. According to current opinion, the tone of the peripheral vascular bed is governed by a bulbar vasoconstrictor centre. Vasoconstriction and vasodilatation can be induced by an increase or decrease in the activity of this centre, reflected in corresponding fluctuations in the intensity of the vasoconstrictor discharges. It is also commonly considered that the oblongate medulla contains a vasodilator centre, which regulates the activity of the vasodilator nervous outflows. Changes in the diameter of the peripheral vessels would therefore be the result of a coordinated and reciprocal action of the vasoconstrictor and the vasodilator centres. Consequently, a vasodilatation would be due to an inhibition of vasoconstrictor tone, to an activation of vasodilator nerves or to both.

The vessels of the skeletal muscles in the cat and dog provide examples of a vascular area with a double vasomotor innervation. The sympathetic outflow supplies these vessels with both vasoconstrictor 1nd vasodilator nerves.

In 1916, RANSON and BILLINGSLEY reported that topical electric stimulation in the most distal part of the rhomboid fossa of the cat elicited vasodepressor reactions. The responsive area has subsequently been designated by various authors as "the bulbar vasodilator centre". SCOTT and ROBERTS (1923) observed that vasodepressor reactions could be produced by stimulation within

a somewhat wider area of the rhomboid fossa and introduced the term "depressor area". LINDGREN and UVNÄS (1953 a, 1954 a) studied the peripheral vascular reactions occurring on electric stimulation of the "vasodilator centre". They were able to elicit pronounced vasodilator responses in the vessels of various vascular areas, among them the vessels of the skeletal muscles. All these vasodilatations were shown to be due to inhibition of vasoconstrictor tone.

In a series of investigations, UVNÄS *et al.* (1951, 1952, 1953 a and b) studied the intracerebral organization of the sympathetic vasodilator outflow to the skeletal muscles of the dog and cat. In the oblongate medulla, the outflow was shown to run in the ventrolateral region. Topical stimulation in this area elicited unilateral vasodilatation in the skeletal muscles.

It has also been shown that the sympathetic vasodilator nerves are cholinergic, and that vasodilatation elicited by activation of such nerves can be blocked by atropine. Thus, it is possible to differentiate between vasodilatation produced by activation of the vasodilatator mechanism and that produced by vasoconstrictor inhibition. In the former case the dilator responses are suppressed by atropine, in the latter they are not.

The oblongate medulla is considered to be the area towards which nervous impulses are projected, both from the C. N. S. and from peripheral receptors, and in which they are relayed to efferent vasomotor neurons. It therefore seems reasonable to assume that a study of the bulbar vasomotor control would provide information regarding the functional relation between the two vasodilator mechanisms.

The present paper is intended to contribute to the elucidation of this question.

Method.

The experiments were performed on dogs and cats. Chloralose (70 mg/kg) or dial (40—60 mg/kg) was used as the anaesthetic.

For stimulation in the hypothalamus, a 1—2 sq.cm hole was made in the roof of the skull, using a pair of bone forceps and a dentist's drill. Spongious bleedings were stopped with bone wax.

For stimulation in the oblongate medulla, the occipital bone, the atlanto-occipital membrane and the dorsal surface of the first cervical vertebra were exposed by removing the overlying muscles. The occipital bone was partly removed, and the atlanto-occipital membrane opened by a longitudinal incision. In some of the experiments, the caudal part

of the cerebellum was removed by suction, in order to make the rhomboid fossa accessible for inspection.

Both sinus nerves were exposed and dissected free for stimulation. The vagus nerves were exposed and — as a rule — sectioned in the neck, and the central stump prepared for stimulation. The baroceptor mechanisms in the carotid sinus area were functionally eliminated by section of the sinus nerves, or by occlusion of the common carotids low down in the neck. Artificial respiration was given in most experiments.

Electric stimulation was applied with a bipolar electrode made by cementing together two enamelled nichrome wires, each with a diameter of 0.3 mm. Only 0.5 mm of the tips of the electrodes was uninsulated. The electrodes were oriented by means of the Horsley-Clarke technique or, when the rhomboid fossa was exposed, under visual control. Nerve stimulation was performed with electrodes of the Sherrington type. Rectangular impulses from a stimulator with an output resistance of about 1,000 ohms were used. The duration of each impulse was 2 milliseconds and the duration of each stimulation period 10—15 seconds. As a rule, the frequency was 70 per second and the intensity 1—3 volts.

The blood flow was recorded either with the "open" direct technique described by CLEMENTZ and RYBERG (1949) or, in the later experiments, with the "closed" technique devised by LINDGREN and UVNÄS (1954 b). In principle, the venous outflow was recorded with a photoelectric drop counter directing an ordinate writer. The venous blood was then returned to the jugular vein or into another suitable vein.

In most experiments, the venous outflow was recorded in one or in each of the two femoral veins (in cats) or in the popliteal veins (in dogs). In order to obtain as pure a muscle blood flow as possible, the legs were skinned and the circulation of the paw excluded by tight ligation just above the ankle. The skinned extremity was wrapped in its loose skin to prevent cooling and drying.

Clotting of the blood was prevented by i.v. injections of heparin (0.2 ml/kg of a 5 per cent solution). The blood pressure was measured in the carotid or the femoral artery with a mercury manometer.

In some experiments, cross-circulation was arranged between two animals. The two hind legs of the recipient animal were skinned and the femoral artery and vein of one leg were exposed down to the popliteal area, and their lateral branches ligated. The muscles, vessels and nerves of the thigh, with the exception of the femoral artery and vein and the sciatic nerve, were tightly ligated just above the knee. The circulation of the paw was tied off. Cross-circulation was established by connecting the femoral artery of the donor animal to the distal part of the ligated femoral artery of the recipient animal. The blood from the femoral vein of the perfused limb was directed to the photoelectric drop counter and was then returned to the jugular vein of the donor animal. With the technique used, the cross-perfused part of the limb could be considered as practically lacking vascular connexions with the recipient animal — only the nervous connexion via the sciatic nerve was intact.

Results.

Fig. 1 shows the area of the distal part of the rhomboid fossa within which, according to LINDGREN and UVNÄS (1954), topical electric stimulation is able to produce depressor reactions (A); they are often extremely marked, with a fall in blood pressure up to 100 mm Hg. Underlying these depressor reactions is a

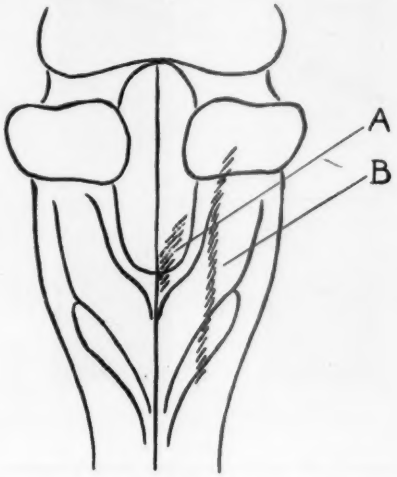

Fig. 1. Schematic drawing of the oblongate medulla, showing the projections on the dorsal surface of the depressor area (A) and the bulbar part of the sympathetic vasodilator tract (B).

general peripheral vasodilatation, at any rate in the skin, the intestines and the muscles. In the intact animal, however, observations of the peripheral blood flow give an unreliable picture of these vasodilator reactions. On haemodynamic grounds, the blood flow cannot increase to any great extent when the blood pressure falls sharply as a result of a disturbance in the balance between the peripheral resistance and the cardiac output. Actually, the blood flow can diminish in an area despite pronounced vasodilatation at this site. This is the case, for instance, in the skeletal muscles. One way of obtaining information on the magnitude of vasodilatation is to keep the blood pressure in the vascular area under observation at a constant level by cross-perfusion. An illustration

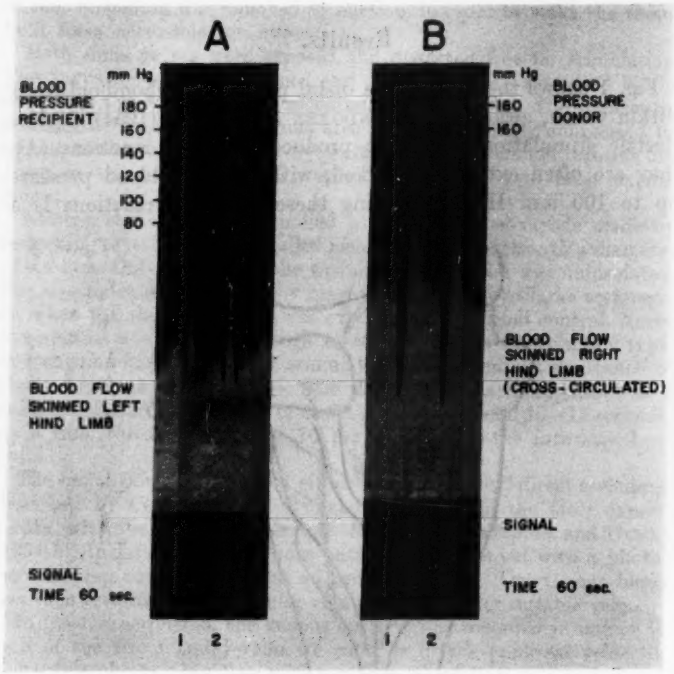

Fig. 2. Cross-circulation. Recipient cat 2.7 kg. Bilateral vagotomy. Both carotids occluded. Donor cat 4.0 kg. Both carotids occluded.

Vasomotor responses in the skinned hind legs to stimulation in the depressor area of the oblongate medulla. Note the increased blood flow in the cross-perfused leg (B) but the decreased flow in the non-perfused leg (A). Voltage 2.0 V. Duration of stimulation 15 sec.

is given in Fig. 2. The muscle blood flow was recorded concomitantly in the two hind legs of a cat. The right leg was cross-perfused from another cat. The marked increase in blood flow in the cross-perfused leg on stimulation in the depressor area of the recipient is in sharp contrast to the reduction of blood flow that simultaneously occurs in the non-perfused leg.

Fig. 1 also shows the position of the bulbar portion of the sympathetic vasodilator outflow (B). The effects of electric stimulation of this outflow have been described and discussed by LINDGREN and UVNÄS (1953 b).

Systematic exploration, millimeter by millimeter, of the distal

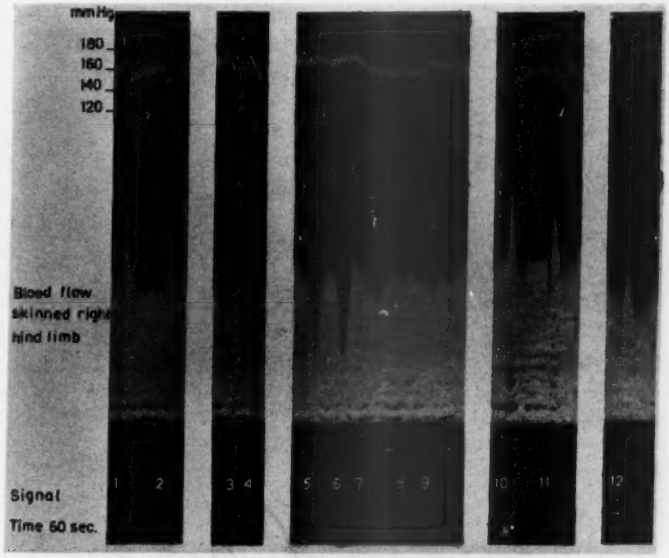

Fig. 3. Cat 2.7 kg. Vasomotor reactions in the skinned right hind leg to stimulation at various points in the oblongate medulla.

1 and 2: Stimulation in the depressor area, 0.5 mm to the right of the midline and 1 mm below the surface. Voltage 1.0 V.

3 and 4: During stimulation, the electrode is forced through the oblongate medulla, 3 mm to the right of the midline in the dorsoventral direction to its ventral surface. Voltage 2.0 V.

5 and 6: Activation of sympathetic vasodilators by stimulation at a point 4 mm to the right of the midline and 2 mm above the ventral surface. Voltage 1.5 V.

7: Atropine 0.1 mg/kg i.v.

8 and 9: Stimulation at the same point as in 5 and 6. Voltage 1.5 V in 8, 2.0 V in 9.

10 and 11: Stimulation in the depressor area at the same point as in 1 and 2. Voltage 1.0 V.

12: Stimulation at the same point, after bilateral occlusion of the common carotids. Voltage 1.0 V.

third of the rhomboid fossa from its dorsal down to its ventral surface revealed a vasodilatator "silent" zone between the depressor area and the sympathetic vasodilator outflow. Within this zone, no points were found which, on stimulation, yielded vasodepressor reactions or vasodilatations. This fact is illustrated in Fig. 3. In this experiment, stimuli were first applied in the depressor area (1 and 2 in the Figure); this resulted in depressor reactions. The fact that the blood flow simultaneously diminished in the

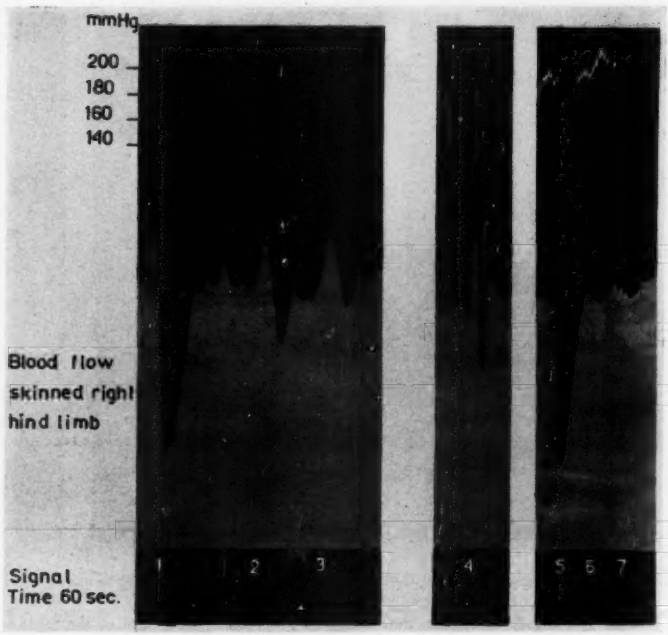

Fig. 4. Cat 3.0 kg. Bilateral vagotomy and occlusion of both carotids.

1. Vasodilator responses in the skeletal muscles due to activation of sympathetic vasodilator nerves by stimulation in the hypothalamus. Voltage 3.0 V.
2. Depressor reaction on stimulation in the depressor area of the oblongate medulla. Voltage 2.0 V.
3. Depressor reaction on stimulation of the left vagus nerve. Voltage 2.0 V.
4. After bilateral cauterization of the depressor area, stimulation of the left vagus nerve gives a pressor response.
5. Stimulation in the hypothalamus at the same point as in 1.
6. Atropine 0.2 mg/kg i.v.
7. The same stimulation as in 1 and 5. The vasodilator response is blocked by atropine.

skeletal muscles (in this experiment recorded in the right hind legs) does not exclude a vasodilatation in the muscles. As already pointed out, there is in fact a hidden vasodilatation underlying the decrease in flow.

The electrode was then moved successively in a lateral direction, one mm at a time; in each plane it was pushed down mm by mm from the dorsal to the ventral surface of the oblongate medulla. No significant depressor reactions or vasodilatations were observed until the electrode reached a point 4 mm lateral to the

midline and 2 mm above the ventral surface. At this point, stimulation produced a pronounced increase in the blood flow of the muscles. Attention is drawn to the inappreciable blood pressure reactions that accompanied the responses of the muscle vessels. This stability of the blood pressure is characteristic of the vasomotor responses which occur on stimulation of the sympathetic outflow, and is due to simultaneous vasoconstriction in the skin and the intestines. That the vasodilatation is due to a sympathetic vasodilator discharge is confirmed by the fact that atropinization completely abolished the vasodilator responses (8 and 9 in the Figure). As seen, repeated stimulations in the depressor area still elicited unchanged depressor reactions.

In another type of experiment, the sympathetic vasodilator outflow was activated by hypothalamic stimulation. A marked vasodilatation occurred in the muscles (1 in Fig. 4). Depressor reactions were then induced by stimulation in the depressor area and by stimulation of the central stump of the vagus nerve (2 and 3 in Fig. 4). For the reasons discussed above, the curve for the muscle blood flow does not reflect the simultaneous vasodilatations in the muscles. During the initial phase of the depressor reactions, a transient decrease took place in the blood flow; the subsequent increase occurred only when the blood pressure once more started to rise. Superficial bilateral cauterization was then performed within the distal tip of the rhomboid fossa, using a pin-point sized thermocautery. Repeated vagal stimulation then yielded — in contrast to previous stimulations — a pressor effect. It is worth mentioning that, despite the marked increase in blood pressure, the blood flow diminished during stimulation. This implies a vasoconstriction in the muscles as well. Repeated stimulation in the depressor area was then without effect. On moving the electrode tip a few mm downwards, however, stimulation elicited vasopressor responses. A feature of great interest was that repeated stimulation in the hypothalamus produced unchanged vasodilator responses in the muscles. Thus, the bulbar sympathetic vasodilator outflow was still intact. In other similar experiments, cauterization of the depressor area resulted in the disappearance of both depressor and pressor responses to centripetal stimulation of a vagus nerve. In all the experiments, the sympathetic vasodilator responses to hypothalamic stimulation persisted.

In another series of experiments with the cross-circulation technique, vasodilator responses were produced in the skeletal

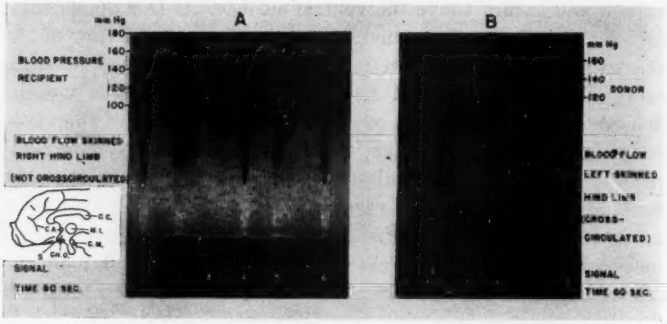

Fig. 5. Cross-circulation. Recipient dog 6.0 kg. Bilateral occlusion of carotids. Donor 3.5 kg.

Vasodilator responses in the skeletal muscles of the non cross-circulated right hind leg (A) and the cross-circulated left hind leg (B) to stimulation in the hypothalamus (S in the schematic drawing to the left in the Fig.) and response to stimulation of the right sinus nerve.

1, 4 and 6: Stimulation in the hypothalamus. Voltage 2.0 V. Duration 10 sec.
2 and 5: Stimulation of the right sinus nerve. Voltage 2.0 V. Duration 30 sec.
3: Atropine 0.1 mg/kg i.v. to the donor dog. Note that the vasodilator response to hypothalamic stimulation disappears in the cross-circulated leg after atropine to the donor; the vasodilatation due to stimulation of a sinus nerve is unaffected.

muscles both by activation of the sympathetic vasodilator outflow, and by eliciting depressor reactions. Sympathetic vasodilator discharges were elicited by hypothalamic stimulation, and depressor reflexes by afferent stimulation of a sinus or a vagus nerve. A in Fig. 5 illustrates vasodilator responses in the non-perfused leg, and B in the same figure the corresponding responses in the cross-perfused leg.

As shown in the Figure, hypothalamic as well as sinus nerve stimulation elicited vasodilatation in both legs, followed by a fall in blood pressure (1 and 2 in the Fig.). A small dose of atropine (0.1 mg/kg) to the donor animal sufficed to abolish completely the vasodilator responses to hypothalamic stimulation (4 and 6 and B in the Fig.). The vasodilator responses to sinus nerve stimulation, on the contrary, remained unchanged (5 in B in the Fig.). Obviously, both types of vasodilator responses persisted in the non-perfused, and thus non-atropinized, leg (4, 5 and 6 in A in the Fig.). Identical results were obtained in experiments in which depressor reactions were elicited by afferent stimulation of a vagus nerve.

Discussion.

The dominant rôle ascribed to the oblongate medulla in the nervous control of the vascular bed was pointed out in the introduction to this paper. No evidence has been brought forward in the present investigation that directly challenges the validity of this statement. It is, however, justified to stress that other areas in the C. N. S. as well play an important part in the control of the peripheral blood flow. The oblongate medulla seems to be of no, or of only minor, importance for the activity in the sympathetic vasodilator outflow. Consequently, the activity of this part of the vasomotor innervation must be regulated by other central nervous structures. In harmony with this conclusion is the recent observation (LINDGREN 1954) that the sympathetic vasodilator tract runs to and relays in the collicular area. Caudally to the collicular area the vasodilator tract innervates only ipsilateral skeletal muscles.

A commonly held view is that the various vasodilator nervous outflows are under the influence of an integrating centre in the oblongate medulla (BAYLISS, among others). This "vasodilator" centre is considered to be situated in the distal part of the rhomboid fossa. It would thus be identical with the depressor area, stimulation of which has been observed to yield depressor reactions (*e. g.* RANSON and BILLINGSLEY 1916, SCOTT and ROBERTS 1923, ALEXANDER 1946). If this hypothesis were true, it should be possible to elicit activity in vasodilator nerves either by direct stimulation in the depressor area, or by eliciting depressor reflexes through the stimulation of afferent nerves, for instance of a vagus or a sinus nerve. The conceivable vasodilator mechanisms are the afferent fibres in the dorsal roots, postulated to carry antidromic impulses to the skin, the cranial and sacral parasympathetic vasodilator nerves to the tongue, the oral cavity and the genital organs, and the sympathetic vasodilator outflow to the skeletal muscles. In a previous paper (LINDGREN and UVNÄS 1954 a) we have shown that stimulation of the depressor area or of the depressor nerves produced depressor reactions due to simultaneous vasodilatation in the skin, the tongue and the muscles; in other words, precisely in those areas considered to possess the aforementioned vasodilator innervation. However, in none of these vascular areas was the vasodilatation due to vasodilator discharges. It was caused by inhibition of vasoconstrictor tone.

In the present investigation, the depressor area was destroyed by local superficial cauterization. This procedure abolished depressor reactions elicited by sinus or vagus nerve stimulation, but did not influence vasodilator responses to activation of the sympathetic vasodilator nerves by hypothalamic stimulation. Moreover, it was shown that vasodilator effects in the muscles, produced by stimulation in the depressor area, were not abolished by atropine, in doses which completely suppressed vasodilatations of the same magnitude induced by sympathetic vasodilator impulses. These observations are in agreement with the assumption that the medullary part of the sympathetic outflow lacks either anatomical or functional connexions with the depressor area.

Thus, vasodilatation can be produced in the skeletal muscles by two nervous mechanisms. On one hand, sympathetic vasodilator nerves can be activated and, on the other hand, vasoconstrictor nerves can be inhibited. The latter procedure can be initiated either by depressor reflexes or by direct stimulation of the depressor area in the oblongate medulla. The fact that depressor reactions cannot be elicited on stimulation of a sinus or a vagus nerve, after local cauterization of the depressor area, indicates that this area forms a part of the depressor reflex arc. On the other hand, the depressor reactions to afferent stimulation of a peripheral nerve are uninfluenced by destruction of the depressor area (LINDGREN and UVNÄS 1954 a). Since this depressor reflex as well is due to inhibition of vasoconstrictor tone, the depressor area cannot be a general integrative centre for vasoconstrictor inhibition in depressor reflexes. It therefore seems reasonable to assume that the depressor area is functionally involved only in vascular reflexes elicited from the baroceptors in the carotid sinus and the aortic regions.

As already mentioned, the sympathetic vasodilator outflow appears to be entirely unassociated with blood-pressure regulating mechanisms. In earlier papers (LINDGREN and UVNÄS, 1953 and others) it has been suggested that this vasodilator system takes part in the regulation of the muscle blood flow during exercise. Among present observations in support of this hypothesis is the close anatomical connexions between the sympathetic vasodilator pathways and the pyramidal tract. The sympathetic vasodilator neurons originate in the motor cortex (ELIASSON *et al.* 1952), and in the oblongate medulla they are found to run in close proximity to the bulbar motor pathways.

Summary.

Vasodilatation in the skeletal muscles is produced by electric stimulation of a sinus nerve, a vagus nerve, the depressor area of the oblongate medulla or by activation of sympathetic vasodilator nerves.

Destruction of the depressor area does not prevent the transmission of impulses in the bulbar sympathetic vasodilator tract.

The depressor area and the bulbar part of the sympathetic vasodilator tract seem to be separate anatomical and functional entities. The writers have been unable to activate the two vasodilator mechanisms simultaneously, a fact that is regarded to support the conception that they form two independently operating mechanisms.

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Effect of Vestibular Nerve Section Upon the Spinal Influence of the Bulbar Reticular Formation.

By

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Angular stimulation of the optimally positioned horizontal semicircular canals gives rise to increased flexor activity combined with inhibited extensor activity, as demonstrated by recording the efferent impulse stream from peripheral motor nerves of the cat (GERNANDT 1952) or the reflex compensatory muscular reactions of the pigeon (VAN EYCK and GERNANDT 1953). These effects, obtained by rotation towards the recording side, were reversed when angular stimulation was applied in the opposite direction. This is in accordance with the vestibular influence, when adequate stimulation is used, upon the peripheral nerve to ventral root reflexes as described by GERNANDT and THULIN (1953). Notably, however, these findings are the direct opposite of those seen following electrical stimulation of the vestibular nuclei. By the latter, the extensor reflexes are facilitated (SPRAGUE, SCHREINER, LINDSLEY and MAGOUN 1948, THULIN 1953, GERNANDT and THULIN 1954), and unilateral destruction of the vestibular nuclei diminishes the extensor tonus on the same side (SPIEGEL 1927, FULTON, LIDDELL and RIOCH 1930, SPRAGUE and CHAMBERS 1953). The apparent incompatibility of these results — all of them readily verified — must be dependent upon the existence of a difference between the effects of electrical stimulation of the vestibular nuclei, or of their extirpation, and those of adequate

vestibular stimulation. The former procedures would seem to be too imprecise to permit satisfactory analysis of the delicate and complex reflex pattern evoked by adequate stimulation. While ampullopetal cupular deviation in the horizontal semicircular canal augments the afferent discharge in the 8th nerve, ampullofugal deviation inhibits it. Similar events in the vertical semicircular canals have the opposite effect (ADRIAN 1943). Also the compensatory muscular responses to identical stimulatory procedures of the different semicircular canals are wholly dissimilar. It is justifiable to ask, therefore, how it is that unilateral electrical stimulation of the vestibular nuclei can have effects resembling those associated with adequate vestibular stimulation.

A spontaneous stream of impulses always traverses the vestibular nerve, even when the animal is in a state of absolute rest, and impinges upon the vestibular nuclei (LOWENSTEIN 1937, LOWENSTEIN and SAND 1940 (a and b), ADRIAN 1943, GERNANDT 1949, ECKEL 1954). These nuclei are interconnected both with the reticular formation on the same side, and with the corresponding nervous structure on the opposite side (GERNANDT and THULIN 1952). The object of the present investigation is to study the question whether section of the ipsilateral and/or contralateral vestibular nerves modifies the inhibited or facilitated spinal motor activity evoked by electrical stimulation of the bulbar reticular formation (cf. MAGOUN 1950, LINDSLEY 1952). The effects on the ventral horn cells were tested by means of oscillographically recorded peripheral nerve to ventral root reflexes. It was deemed likely that this procedure would yield data relevant to the significance of the spontaneous afferent stream of impulses traversing the 8th nerve for the motor activity of the spinal cord (KEMPINSKY and WARD 1950).

Methods.

The experiments were performed on cats which were decerebrated during ether anaesthesia. After tracheal cannulation, the occipital bone was removed, the dura incised, and the cerebellum sucked out. This decerebellate preparation permitted easy access to the 8th nerves which could be sectioned as required, and exposed appropriate areas of the brain stem for electrical stimulation. To eliminate any movements provoked by the bulbar conditioning stimulus, the cats were curarized and given artificial respiration.

The electrode used for the conditioning stimulus consisted of a steel needle, insulated except at the tip, whose diameter did not exceed 0.03 mm. The unipolar method was used. A micromanipulator was employed to insert the electrode into the desired portions of the pons and the medulla oblongata. Being of threshold strength or just above (about 0.5 V), the conditioning stimulus was carried out with sinusoidal pulses with a frequency of 60 c. p. s. (GERNANDT and THULIN 1954).

The effects of conditioning stimuli applied to the bulbar part of the brain stem were studied on peripheral nerve to ventral root reflexes. The nerves used were the medial or the lateral branch to the gastrocnemius muscle and the nerve of the anterior tibial muscle. They were placed upon two separate stimulating electrodes. By means of a switch either nerve could be stimulated separately. The limb to be used was denervated. Single shock stimuli of maximal or supramaximal strength were obtained at regular intervals (one per second) and synchronized with the sweep.

The lumbar spinal cord was exposed and the ipsilateral ventral roots (L_7 or S_1) were cut peripherally to be used for recording the test reflex. The recording electrodes were small Ag—AgCl hooks. A capacity-coupled amplifier and a cathode-ray oscillograph were used for the recording.

To prevent drying or cooling of the exposed spinal cord or the peripheral nerves they were protected with liquid paraffin at body temperature.

Results.

Electrical stimulation of the reticular formation has revealed the existence of discrimination of the bulbar reticular formation for reciprocal innervation (THULIN 1953, SPRAGUE and CHAMBERS 1953, GERNANDT and THULIN 1954) and also that the vestibular responses, even when initiated from the reticular level, are organized for reciprocal action on flexors and extensors (GERNANDT 1952, GERNANDT and THULIN 1953). Stimulation in the medial reticular formation may, for example, have the effect illustrated in Fig. 1, showing its reciprocal action on the reflex responses derived from the gastrocnemius nerve and the nerve to the anterior tibial muscle and recorded from the corresponding ventral root. As will be seen, the reflex response of the extensor nerve (B) was markedly inhibited while the bulbar region was stimulated compared with the control response (A). The effect upon the reflex response elicited from the flexor nerve was the direct opposite of this: bulbar stimulation was then clearly facilitatory (B: 1) when the control response (A: 1) is regarded as the norm. The ipsilateral 8th nerve was now transected. Both 8th nerves having been

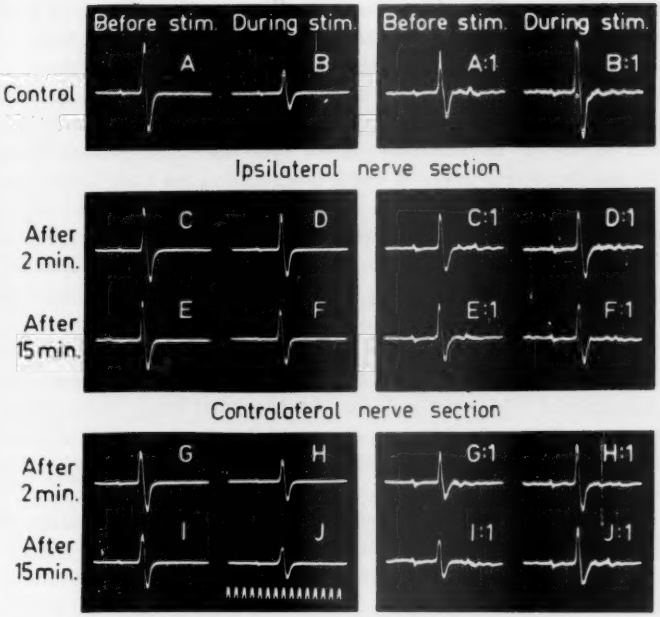

Fig. 1. Decerebrated cat. Records from the ventral root L_7. Action of ipsilateral and contralateral section of the 8th nerves on the monosynaptic test volleys obtained from the gastrocnemius nerve (A to J) and from the nerve of the tibialis anterior (A: 1 to J: 1) before and during bulbar stimulation. Time scale in msec.

exposed initially this could be done without in any way disturbing either the stimulating electrode or adjacent nervous tissue. Sectioning of the nerve caused a negligible haemorrhage from the internal auditory artery but it stopped quickly. Bulbar stimulation now affected the reflex responses differently, as the ipsilateral, afferent vestibular impulse stream was no longer present. The inhibition and facilitation of the reflex responses previously so manifest had thus become far less obvious. The control responses (C and C: 1) were of smaller amplitude. Not necessarily an immediate consequence of such neurotomy, this diminution may become increasingly pronounced over a period of several minutes (KEMPINSKY and WARD 1950, GERNANDT 1952, GERNANDT and THULIN 1953). Generally this gradual reduction comes to an end within 10 to 15 minutes, however, whereupon the amplitude of the control response may remain constant. The reflex responses

were ordinarily 20 to 40 per cent smaller now than they had been before neurotomy. The responses recorded 2 minutes after transection of the ipsilateral 8th nerve are reproduced in C (the gastrocnemius nerve) and C: 1 (the nerve to the anterior tibial muscle) of Fig. 1 and the action of bulbar stimulation appears in D and D: 1. Evidently the inhibitory effect upon the reflex response from the gastrocnemius nerve is much less pronounced than before the neurotomy (compare D with B). The same thing is shown in F, where 15 minutes had elapsed after cutting the 8th nerve. A similarly reduced facilitation of the reflex response provoked from the flexor nerve during bulbar stimulation is illustrated in D: 1 and F: 1.

A further considerable reduction of the amplitude of the control responses (G and G: 1) took place when, at this stage, the contralateral 8th nerve was cut. On the other hand, bulbar stimulation now was again accompanied by a more marked inhibition (H and J) or, respectively, facilitation (H: 1 and J: 1) of the reflex responses.

The results of these experiments indicate that elimination of the ipsilateral inflow of impulses in the 8th nerve makes it impossible to induce equally strong facilitation and inhibition by bulbar stimulation. These impulses normally arrive at the vestibular nuclei which, it has been surmised, participate in the facilitatory function of the brain-stem reticular formation (Ward 1947, Sprague, Schreiner, Lindsley and Magoun 1948, Schreiner, Lindsley and Magoun 1949). However, as a result of more detailed studies, it has become apparent that this facilitation benefits the extensor activity only, while the flexor activity is inhibited (Thulin 1953, Gernandt and Thulin 1954). It should be noted that the latter results were gained by electrical stimulation of the vestibular nuclei themselves. From these nuclei the vestibulo-spinal tract takes its origin and serves to place the motor pool of the spinal cord under the reflex control of the vestibular apparatus. They have, moreover, direct as well as crossed connections with the reticular formation (Papez 1926, Lorente de Nó 1933, Buchanan 1937, Gernandt and Thulin 1953) and fibres, constituting the reticulo-spinal tract, have been traced from here into the spinal cord.

The spontaneous bilateral inflow of impulses into the 8th nerves is unlikely to have much significance for the spinal reflex activity as long as the animal is in a state of absolute rest, for then the

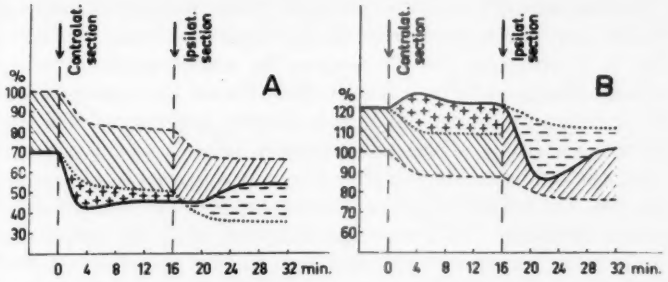

Fig. 2. Action of contralateral and ipsilateral section of the 8th nerves on the monosynaptic test volleys obtained from the gastrocnemius nerve (A) and from the nerve of the tibialis anterior (B) before and during bulbar stimulation. Ordinate: amplitude of the monosynaptic spike as a percentage of the control test response. Abscissa: time in minutes. The broken line delineates the amplitude of the test response after section of the 8th nerves. The dotted line indicates the amplitude during bulbar stimulation that would have been obtained by the reflex volley had not section of the 8th nerves influenced the effect. The solid line, presenting amplitude variations of the reflex volley during bulbar stimulation, represents the true effect.

A: The effect of bulbar stimulation on the reflex response evoked from the gastrocnemius nerve is, in this case, inhibitory, increasingly so following contralateral section of the 8th nerve (+). Part of the incremental inhibition is lost when the ipsilateral nerve is transected too (—).

B: Similar bulbar stimulation facilitates the reflex response from the nerve supplying the anterior tibial muscle. This facilitation is enhanced following section of the contralateral 8th nerve (+). Part of the incremental facilitation is lost after neurotomy of the ipsilateral 8th nerve (—).

effects transmitted via the reticular formation may be neutralized (GERNANDT 1952). The activity conducted via the vestibulospinal pathways, which are of little importance for the spinal reflexes in the cat (BACH and MAGOUN 1947, GERNANDT and THULIN 1953), is identical. Only after the 8th nerve on one side has been severed will the balanced condition of the reticular formation be upset. Then, supposing the ipsilateral nerve is subjected to transection, in conformity with the results of Fig. 1, the activity in the contralateral nerve will have a facilitatory influence on the reflex response of the extensor nerve at the same time as the flexor nerve's reflex response is inhibited. Hence the contrary effect previously accompanying bulbar stimulation — inhibition of the reflex response provoked from the gastrocnemius nerve combined with facilitation of that derived from the nerve of the anterior tibial muscle — would become less marked. Not until the contralateral nerve as well has been transected will the effect of bulbar stimulation become manifest once more.

The outcome of a similar experiment where the order of severing the 8th nerves was reversed is shown diagrammatically in Fig. 2. Fig. 2 A represents the effect upon the monosynaptic extensor reflex discharge and Fig. 2 B the influence upon the corresponding flexor reflex discharge. Taking the control response as 100, the relative amplitude of the monosynaptic reflex volley is set out along the ordinate. Elapsed time in minutes is marked off on the abscissa. The broken line shows the amplitude of the monosynaptic control response. The dotted line indicates what the magnitude of the reflex response would have been had not the effect on it of bulbar stimulation undergone so manifest an alteration following transection of the 8th nerves. The true effect is delineated by the solid line, demonstrating the influence upon the monosynaptic reflex volley's amplitude as a result of bulbar stimulation. In this experiment the stimulating electrode was also applied in the medial reticular formation, and the reciprocal effects produced thereby appeared similar in all respects to those in Fig. 1, viz. facilitation of the reflex response elicited from the gastrocnemius nerve and inhibition of the reflex discharge from the nerve to the anterior tibial muscle. The contralateral and ipsilateral nerve sections are marked by the arrows. The monosynaptic test response (broken line) displayed the usual gradual diminution over a period of minutes following the section of the 8th nerves.

The inhibition during bulbar stimulation of the reflex response elicited from the extensor nerve will be found to the left in Fig. 2 A. During similar stimulation after the contralateral 8th nerve had been cut this inhibition was even further accentuated (+). But subsequent transection of the ipsilateral nerve will bring about a reduction of the effect, making the inhibition due to bulbar stimulation much less marked comparatively speaking. Rather than lowering the amplitude of the monosynaptic reflex response to the level of the dotted line, the inhibitory action of bulbar stimulation could then only lower the amplitude to values marked by the solid line. Consequently ipsilateral neurotomy was accompanied by a loss of inhibitory effect (−).

In Fig. 2 B the reflex response was evoked from the nerve to the anterior tibial muscle and the facilitation due to bulbar stimulation is shown to the left. Severance of the contralateral nerve accentuated this facilitatory effect (+), but the increment was again lost when the ipsilateral 8th nerve was cut off (−).

In addition to regions within the bulbar reticular formation

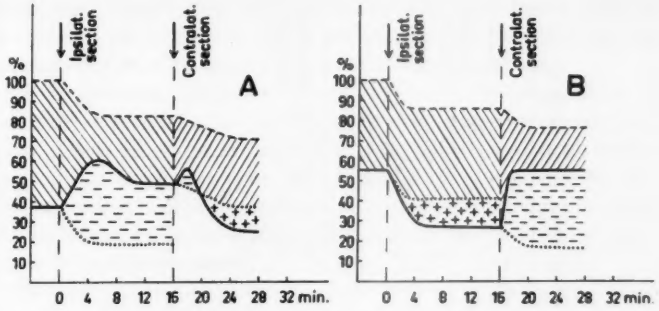

Fig. 3. Action of ipsilateral and contralateral section of the 8th nerves on the monosynaptic test volleys obtained from the gastrocnemius nerve (A) and from the nerve of the tibialis anterior (B) before and during stimulation of a region within the bulbar reticular formation giving a non-reciprocal inhibition. For further explanations see legend to Fig. 2.

where stimulation brings about clear-cut reciprocal effects on the two-neuron stretch and on multi-neuron flexor reflexes recorded from the ventral root (GERNANDT and THULIN 1954), there are other anatomically separable portions of the reticular formation where, as shown by MAGOUN and co-workers (MAGOUN 1950), stimulation gives a non-reciprocal inhibition or facilitation. The diagram in Fig. 3 depicts the effects displayed by the monosynaptic reflex responses provoked from the flexor and extensor nerves following transection of the 8th nerves. Bulbar stimulation was now accompanied by indiscriminate inhibition of both these reflex responses. The effect on the reflex discharge elicited from the extensor nerve appears on the left in Fig. 3 A. After sectioning the ipsilateral 8th nerve the inhibitory effect of bulbar stimulation was markedly less than when the afferent impulses were transmitted by an intact nerve (—). Then the contralateral nerve was cut. However, disregarding some variation initially (—), an effect occasionally seen for some minutes immediately following transection of the 8th nerve, the inhibitory effect was again accentuated when the contralateral nerve was cut off (+).

Analysis along similar lines of the effect on the flexor reflex discharge (Fig. 3 B) revealed that partition of the ipsilateral nerve brought about a slightly greater inhibition (+) during bulbar stimulation. But elimination of the contralateral nerve took away once more all that had been gained (—). Evidently, transection of the 8th nerves tends to influence reciprocally the reflex responses.

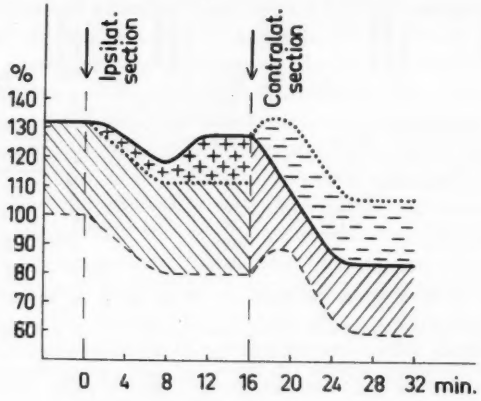

Fig. 4. Action of ipsilateral and contralateral section of the 8th nerves on the monosynaptic test volleys obtained from the gastrocnemius nerve before and during stimulation of a region within the bulbar reticular formation giving a non-reciprocal facilitation. The opposite effect applies to the reflex response derived from the flexor nerve (cf. Fig. 1, A: 1 to J: 1). For further explanations see legend to Fig. 2.

When the stimulatng electrode is so located in the reticular formation that both ireflex responses are facilitated during stimulation, ipsilateral neurotomy will enhance still more the amplitude of the reflex response evoked from the extensor nerve. Most of the facilitatory effect will be lost, however, when the contralateral nerve is eliminated (Fig. 4). The opposite applies to the reflex response derived from the flexor nerve (cf. Fig. 1). That is, the response, initially facilitated owing to bulbar stimulation, will become far less facilitated following ipsilateral transection of the 8th nerve, but is again slightly enhanced when the contralateral nerve has been cut too.

Discussion.

Before proceeding to discuss conceivable mechanisms that might underlie these findings, which are in accordance with the results of adequate vestibular stimulation, we shall recapitulate the following experimental data.

(i) Ampullopetal cupular deviation in the ampulla of the horizontal semicircular canal either exerts an ipsilateral facilitatory action on the reflex response evoked from a flexor nerve or aug-

ments the efferent flow of impulses in that same nerve. This is combined with inhibition of the reflex response derived from an extensor nerve or an inhibitied efferent impulse stream.

(ii) Remaining unaffected when the vestibulo-spinal tract is cut, this reciprocal effect is probably mediated by the reticulo-spinal tract. In addition descending fibres composing the medial longitudinal fasciculus take their origin in the vestibular nuclei. Yet, according to the literature, this pathway is unlikely to wield any influence upon the spinal motor pool at the level the present paper is concerned with.

The facilitation or inhibition of the reflex responses by bulbar stimulation is transmitted over reticulo-spinal fibres. Moreover, both ipsilaterally and contralaterally, the reticular formation connects with the vestibular nuclei. Accordingly some of the fibres in this tract will transmit activity to the motor pool from the vestibular nuclei of the same side and others will mediate activity from nuclei on the other side. Apparently, however, activity in these fibres has opposite effects on the spinal reflexes elicited from extensor nerves and from flexor nerves. These effects do not become manifest, however, so long as the spontaneous impulse discharges in the two 8th nerves are balanced. The effect becomes manifest only when the 8th nerve has been eliminated on one side. If the transected nerve is the ipsilateral one, the reflex response evoked from the gastrocnemius nerve will have its amplitude reduced (Fig. 1). Probably this comes about through cessation of activity in the vestibulo-spinal tract. This tract, however, plays a much less significant rôle in the cat than does the reticulo-spinal tract (Bach and Magoun 1947, Gernandt and Thulin 1953). Nevertheless the possibility cannot be excluded that the spontaneous afferent impulse stream reaching the vestibulo-spinal tract via the vestibular nuclei may exert a small degree of facilitatory action. If now the contralateral nerve is sectioned, the amplitude of the reflex response will display a further reduction. It follows, therefore, that the crossed connections between the vestibular nuclei and the reticular formation transmit facilitatory impulses counteracting the inhibition elicited by electrical stimulation applied to the particular portion of the reticular formation concerned.

But the impulses in the same crossed connection exert an inhibitory action on the reflex response obtained from the nerve supplying the anterior tibial muscle. The facilitation of the reflex

discharge during bulbar stimulation, substantial prior to neurotomy, becomes less marked when the contralateral nerve alone is available to the spontaneous impulse stream. The converse applies to the spontaneous afferent impulse discharge in the ipsilateral 8th nerve. In these circumstances it inhibits the reflex response provoked from the extensor nerve and facilitates that from a flexor nerve.

This assumption is further supported by the results of the experiment presented in Fig. 2. In this experiment the 8th nerves were sectioned in reversed order, viz. the contralateral first and the ipsilateral second. The inhibition of the reflex response from the extensor nerve due to stimulation of the reticular formation became still more inhibited after transection of the contralateral nerve. However, subsequent to partition of the ipsilateral nerve the strong inhibitory effect was again lost. With respect to the reflex response derived from the flexor nerve, the facilitation due to bulbar stimulation became still more accentuated following partition of the contralateral 8th nerve. Subsequent elimination of the ipsilateral 8th nerve caused much of this facilitation to be lost.

From these experiments and earlier investigations involving the use of adequate vestibular stimulation, it is evident that the impulse stream existing in the 8th nerve and transmitted via the reticular formation exerts a reciprocal influence on the ventral horn cells. Merely the elimination of the spontaneous impulse activity present in the 8th nerves when the animal is at rest is enough to produce these effects.

Summary.

Electrical stimulation within the reticular formation gives rise to reciprocal or non-reciprocal effects upon the spinal reflexes evoked from extensor and flexor nerves. The present investigation was designed to analyse the behaviour of this caudal influence of the bulbar reticular formation after transection of the 8th nerves. Cessation of the spontaneous afferent impulse discharge in the ipsilateral and/or contralateral 8th nerves affects the reflex responses in a manner resembling that of adequate vestibular stimulation. The afferent impulses in the 8th nerve thus facilitated the reflex response evoked from an ipsilateral flexor nerve and inhibited the reflex discharge derived from an ipsilateral extensor

nerve. An increased impulse activity in the contralateral 8th nerve had the opposite effects, namely inhibition of the former reflex response and facilitation of the latter one.

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The Influence of Potassium on the Intestinal Absorption.

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During some experiments on the influence of the pH of the solution infused it was observed that the sodium net-absorption in the distal part of the small intestine, and to a lesser extent in the colon, decreased when using a 1/15 N potassium phosphate buffer compared with the net-absorption when a 1/15 N sodium phosphate buffer or a pure sodium chloride solution was used. This problem has been further investigated in the following experiments.

Methods.

The experiments were made on white rats in urethane anaesthesia. The distal 20 cm of the small intestine and the colon from the ileocaecal junction to the rectum were isolated separately. Each segment was cleaned with an isotonic glucose solution, and subsequently by insufflating air through the segment. In each of the isolated segments 2 ml of a mixture of 0.55 % NaCl and 0.45 % KCl were infused. After an absorption time of 60 minutes the animal was killed, and the content of each segment analyzed separately.

Results.

The results are shown in table I. In the *colon* the sodium net-absorption was on an average 37.6 % of the infused amount, and the chloride net-absorption 57.4 %, while in the *small in-*

Table I.

Colon						Small Intestine					
Sodium		Potassium		Chloride		Sodium		Potassium		Chloride	
Absorbed in %	End-Concentration	Absorbed in %	End-Concentration	Absorbed in %	End-Concentration	Absorbed in %	End-Concentration	Absorbed in %	End-Concentration	Absorbed in %	End-Concentration
49.9	0.11	48.9	0.11	62.9	0.19	31.0	0.29	81.7	0.08	61.4	0.39
31.0	0.13	23.0	0.14	48.4	0.22	26.9	0.23	75.2	0.08	51.3	0.37
22.7	0.12		0.15	51.3	0.17	30.0	0.27	80.0	0.08	64.1	0.33
39.0	0.12	41.5	0.12	58.1	0.20	63.6	0.31	91.3	0.08	85.5	0.29
10.6	0.18	42.4	0.12	46.7	0.26	37.7	0.28			68.1	0.34
72.2	0.08	38.5	0.19	77.1	0.16	41.8	0.25	81.9	0.08	62.0	0.39
37.6	0.12	38.9	0.14	57.4	0.20	38.5	0.27	82.0	0.08	65.4	0.35

Table I shows the net-absorption and the end-concentrations of sodium, potassium and chloride ions after infusion of a mixture of 0.55 % NaCl and 0.45 % KCl.
Infused volume 2 ml, absorption time 60 minutes, end-concentrations in %.

testine it was 38.5 % and 67.5 %. Previously (BUDOLFSEN 1954) it has been found in experiments with the same sort of animal, the same experimental methods, and the same intestinal segments that the net-absorption of sodium amounted on an average to 43.7 % in the colonic segment, and 67.5 % in the ileal segment when a sodium chloride solution with the same sodium concentration as the sodium-potassium chloride mixture was infused. The chloride net-absorption in 6 experiments was on an average 60.4 % in the colon, and 67.1 % in the ileal segment when a pure sodium chloride solution with the same chloride content as the mixture was infused.

In the colon the addition of potassium chloride has not influenced the net-absorption of sodium as well as chloride significantly. In the ileal segment, however, the sodium net-absorption decreases from averagely 67.5 % to 38.5 % by the addition of potassium chloride. The decrease is statistically significant. The chloride net-absorption in the ileal segment is uninfluenced by the addition of potassium chloride.

The potassium net-absorption was on an average 38.9 % of the amount infused in the colon, and 82 % in the ileal segment, so that the absorption rates of sodium and potassium are of the same order in the colon, while potassium is absorbed much faster than sodium in the ileum.

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The water net-absorption calculated on the basis of the end-concentrations and the not-absorbed amount is uninfluenced by the addition of KCl. The end-concentrations of sodium and chloride are also uninfluenced by the potassium addition.

The only potassium effect observed is thus a decrease of the sodium net-absorption in the ileum. This effect has not been observed before, but a similar result has been found in the kidneys (BOCK 1907, MILLER 1926, KRUHØFFER 1950).

GARDNER and BURGET (1938) have observed that the addition of potassium to a glucose solution influences the intestinal glucose absorption. Experiments on isolated dog intestinal segments showed that the addition of 0.1 % KCl increased the glucose absorption with 10 %, and in experiments on rats when the Cori method was used, it was demonstrated that an increase from 0.25 to 0.50 % of the potassium chloride concentration of the glucose solution infused increased the glucose net-absorption with 2 %. These results are, however, highly insignificant.

Therefore I have further investigated the influence of potassium on the glucose net-absorption.

Methods.

The experiments were made with the same methods as the aforementioned with the following modifications: the cleaning solution was a 0.9 % sodium chloride solution, the infused solution was either a pure 2.7 % glucose solution or a mixture composed of 2.7 % glucose with KCl or NaCl, and the absorption time was 150 minutes.

Results.

The results are seen in table II. By adding 0.45 % KCl to a 2.7 % glucose solution a statistically significant decrease of the glucose net-absorption is observed in the ileal segment, but not in the colon. The addition of NaCl to the glucose solution infused does not influence the glucose absorption, so that it cannot be an effect of the chloride ion.

Discussion.

The decrease of the sodium net-absorption in the ileal segment by adding potassium to the solution infused brings to memory the well known experiments of CORI (1925/26) in which it was

Table II.

Solution infused		Glucose Absorption in %	
		Colon	Small Intestine
2.7 % glucose + 0.45 % KCl		34.7 %	48.8 %
		14.4 »	82.4 »
		18.7 »	50.2 »
		29.3 »	77.2 »
		19.9 »	55.4 »
		14.6 »	65.1 »
	Average	21.9 %	63.2 %
2.7 % glucose + 0.45 % NaCl		29.4 %	100.0 %
		19.6 »	100.0 »
		25.4 »	68.0 »
		26.4 »	100.0 »
		34.6 »	100.0 »
	Average	27.1 %	93.6 %
2.7 % glucose		38.9 %	89.4 %
		29.6 »	99.1 »
		24.3 »	76.1 »
		11.1 »	96.7 »
		13.0 »	95.7 »
		16.9 »	96.7 »
		39.1 »	80.0 »
		54.4 »	74.2 »
		18.6 »	90.9 »
	Average	27.3 %	88.8 %

Table 2 shows the glucose net-absorption in % from different glucose solutions. Absorption time 150 minutes. Infused volume 2 ml.

observed that the addition of galactose to a glucose solution decreases the absorption rate of glucose. This observation is generally explained as galactose occupying a part of the active absorption mechanism for glucose. Against such an explanation of the potassium effect on the sodium net-absorption speaks the observed potassium effect on the glucose absorption, unless we assume that glucose and sodium are absorbed by the same mechanism in the ileum, but we have no experimental basis for such a theory. Some facts speak against it, viz. that in the colon of white rats an active absorption mechanism for sodium chloride but not for glucose have been observed (BUDOLFSEN 1952), and that the addition of sodium to a glucose solution does not influence the intestinal glucose absorption.

Therefore it seems more likely that the influence of potassium is an unspecific effect on the ileal mucosa, while the colonic mucosa

is more resistent. The difference is possibly caused by the smaller net-absorption of potassium in the colonic than in the ileal segment.

Summary.

In experiments on white rats it has been observed that the addition of potassium to solutions of glucose and sodium chloride causes a decrease of the glucose and the sodium net-absorption in the distal part of the small intestine, but not in the colon. The effect is supposed to be caused by an unspecific effect on the mucosa.

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Reproduction of the 3 per Second Spike and Wave EEG Pattern by Subcortical Electrical Stimulation in Cats.

By

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Introduction.

In 1941 JASPER and KERSHMAN (see also JASPER 1941) proposed the theory of a diencephalic pace-maker mechanism, underlying the 3 per second bilaterally synchronous spike and wave EEG pattern (GIBBS, DAVIS and LENNOX 1935) in petit mal epilepsy in man. JASPER and DROOGLEEVER-FORTUYN (1947) later demonstrated that cortical potentials, similar to the petit mal EEG pattern, could be produced in cats by electrical stimulation of thalamic structures adjacent to the massa intermedia. HUNTER and JASPER (1949) extended the studies in stimulation experiments in unanesthetized cats with implanted electrodes, and they demonstrated parallels — in EEG and behaviour — between cats, electrically stimulated in medial thalamic areas, and patients during petit mal seizures. The clinical implications of these experimental findings have been discussed by PENFIELD and JASPER (1947; see also JASPER 1949 a and b and PENFIELD 1949, 1952). Recently GIBBS and GIBBS (1952) and PENFIELD and

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JASPER (1954) have thoroughly reviewed the present knowledge of the petit mal epilepsy and its pathophysiology.

The present investigation was carried out in order to study the detailed localization and the extent of the subcortical structures from which typical spike and wave potentials may be elicited by subcortical electrical stimulation in cats. It was also attempted to clarify the experimental conditions (see JASPER and DROOGLEEVER-FORTUYN 1947, p. 288) under which such cortical responses may appear.

Technique.

The experiments were carried out in 32 cats, mainly under nembutal anesthesia (35 mg/kg). Preparations of the *encéphale* or *cerveau isolé* type were also used as well as unanesthetized ones, immobilized with curare. The hemispheres were usually widely exposed, and the cortical surface was protected with mineral oil.

Stimulation was carried out with stereotaxically oriented electrodes — of the type described by JASPER and DROOGLEEVER-FORTUYN (1947) and by HANBERY, AJMONE-MARSAN and DILWORTH (1954) — approaching subcortical structures horizontally through the posterior fossa or perpendicularly from above. Square wave stimuli were delivered from a Grass stimulator employed in connection with a radio frequency output isolation unit. Many parameters of stimulation were tested. Single shocks, repeated at low frequencies (1 to 3 per second), were mostly used, occasionally higher frequencies of up to 250 c/s. With "concentric" bipolar electrodes it was necessary to employ a stimulation intensity of 10 to 25 V, with bipolar needle electrodes 4 to 20 V. The pulse duration varied between 0.5 and 2 msec.

About 4,000 points were stimulated which covered subcortical structures between the interauricular plane and a frontal plane 15 millimeters rostral to it. In attempts to obtain bilateral cortical effects, structures within 5 millimeters from the midline were specifically explored. The stimulation points were identified in serial sections of the brain (Nissl and Weil stains) in which the electrode positions were marked by electrolytic deposition of iron, later stained by potassium ferrocyanide in the fixation fluid.

Recording was made from the cortical surface with small silverwire bipolar electrodes with a 3 millimeter separation between their rounded tips. The potentials recorded were fed to 6 modified Offner EEG condensor coupled amplifiers, used with a time constant between 0.2 to 0.5 seconds. Paper records were obtained with an ink-writer, and photographs were also taken from a 5-beam oscilloscope. With "monopolar" recording the reference electrode was placed on the occipital muscles.

Fig. 1. EEG record of cortical responses (recorded with symmetrically placed bipolar surface electrodes) to stimulation of n. centralis medialis at frontal plane + 9 (3 per second, 10 V, 2 msec. pulse duration). Nembutal preparation.

Results.

The experimental findings of JASPER and DROOGLEEVER-FORTUYN (1947) in cats (nembutalized or of the *encéphale* or *cerveau isolé* type) were confirmed by the following findings:

1. It was possible to elicit cortical spike and wave patterns by stimulation of the massa intermedia or structures in its immediate vicinity (fig. 1). The yield of typical potentials was, however, very small in comparison to the great number of stimulations carried out.

2. With electrodes of the type used, the stimulation voltage necessary to elicit the spike and wave pattern was found to be high (as a rule more than 10 V, fig. 2). The impression was obtained that deep nembutal anesthesia blocked typical responses and that they were more frequent under unanesthetized conditions.

3. A long-lasting "self-sustained" 3 per second spike and wave activity was never produced by any form of stimulation, except as one or two 3 per second slow waves, similar to those described by JASPER and DROOGLEEVER-FORTUYN (1947, fig. 89). In order to maintain the 3 per second rhythm — in experiments in which such potentials occurred — it was found necessary to repeat the shocks at a rate of 3 per second (fig. 3).

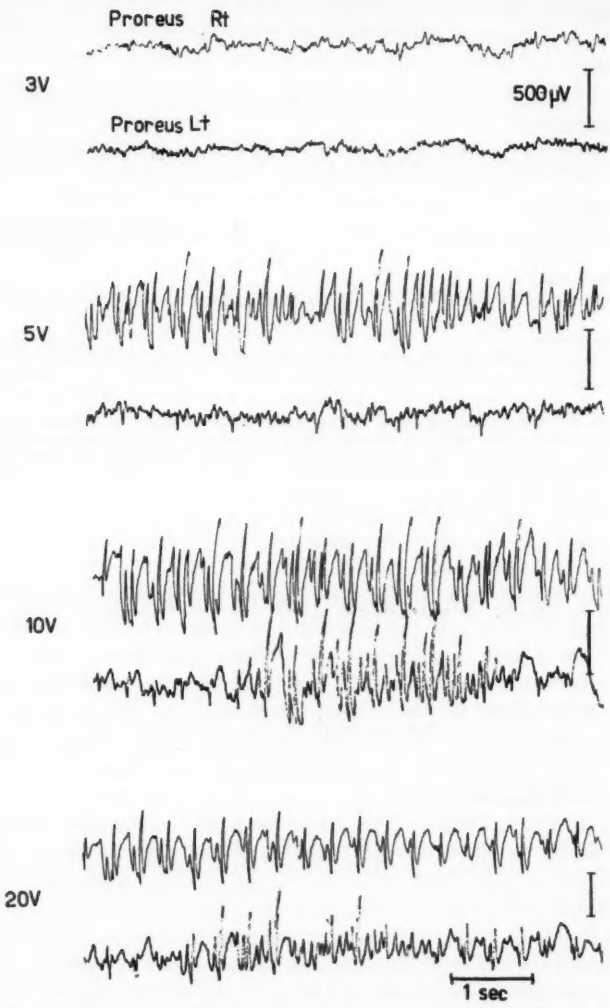

Fig. 2. Frontal cortical responses to thalamic stimulation at 3 per second with increasing voltage. Stimulation of border between n. medialis dorsalis and n. centralis medialis 2 to 3 millimeters from the midline on the right side. Monopolar recording (negative up). *Encéphale isolé* preparation.

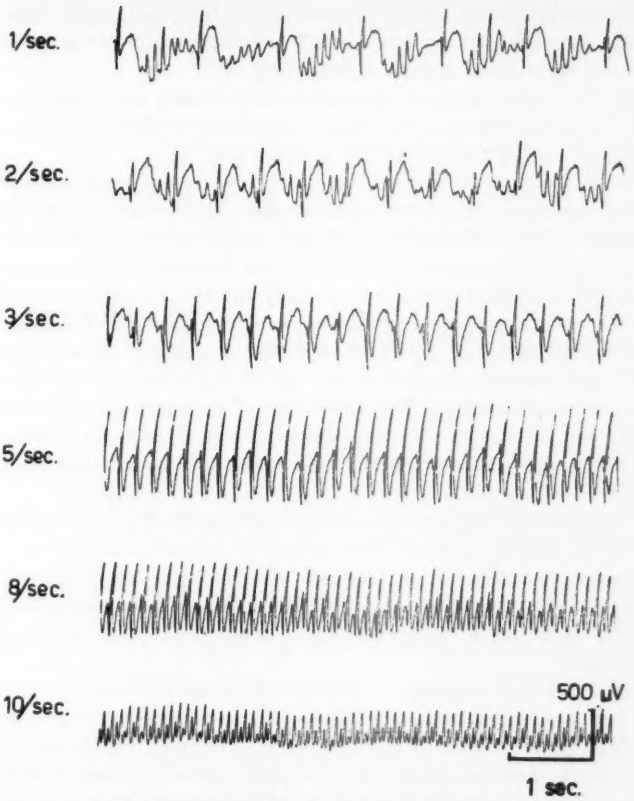

Fig. 3. Frontal cortical responses on stimulating n. centralis medialis with increasing frequencies (25 V, 1.1 msec. pulse duration). Monopolar recording (negative up). *Encéphale isolé* preparation.

In a few instances, however, high frequency (200 c/s) stimulation of subthalamic regions produced long lasting (around 30 seconds) trains of wide-spread synchronous slow waves in the EEG from the "delta" band (frequencies between 0.5 to 3.5 per second). This finding confirms observations by MORISON, FINLEY and LOTHROP (1943) and by JASPER (1952). It contrasts to the more usual result of such stimulation in this region, *i. e.*, activation of the EEG (MORUZZI and MAGOUN 1949).

4. When produced by a suitable form of stimulation, the 3 per second spike and wave potentials were found to have a pre-

dominantly frontal cortical spread, *i. e.*, they were only seen in gyrus proreus and in immediately adjacent parts of the cortex. Larger areas were seldom included (fig. 1).

5. The spike and wave potentials were usually asymmetrical in the two hemispheres even when stimulating structures in the immediate vicinity of or at the midline (fig. 2).

6. It was confirmed that the conditions are not identical under which the spike and wave response and the "recruiting response" (DEMPSEY and MORISON 1942 and 1943) appear in the cortex. Only when stimulating structures of the thalamic reticular system (see below), was it possible to identify the spike-component of the spike and wave response with the recruiting response. The criteria for identification was the latency, the recruiting character at 5 to 8 per second stimulation as well as the wide cortical spread (see references below). The two forms of responses also differed in that the recruiting one could be produced in most experiments, while, as pointed out already, the spike and wave response was only seen in a few.

In spite of numerous attempts at different depths of anesthesia, or with various forms of unanesthetized preparations, it was not possible to develop a preparation which was stable in regard to the cortical potentials looked for. Often the spike and wave formations would only be present for a short period during the experiment.

The cortical response — which could be driven into the "best" 3 per second pattern — consisted of a spike followed by a slow wave (duration 100 to 250 msec., varying voltage). In the majority of cases, however, there was a "silent" period instead of a slow wave (of the same duration about). The cortical potential complex elicited by the shocks usually ended in a repetitive afterdischarge (fig. 3 and 4 A).

When the necessary conditions were present, spike and wave responses were elicited in the cortex (with bilateral spread), also from structures outside those generally assigned to the thalamic "recruiting" or reticular system (MORISON and DEMPSEY 1942; JASPER and DROOGLEEVER-FORTUYN 1947; JASPER 1949 a; STARZL and MAGOUN 1951; STARZL and WHITLOCK 1952; HANBERY and JASPER 1953; VERZEANO, LINDSLEY and MAGOUN 1953; HANBERY, AJMONE-MARSAN and DILWORTH 1954). Frontal spike and wave responses were, for example, elicited from the hippocampus (bilateral responses; fig. 4 B) (see JUNG and TÖNNIES

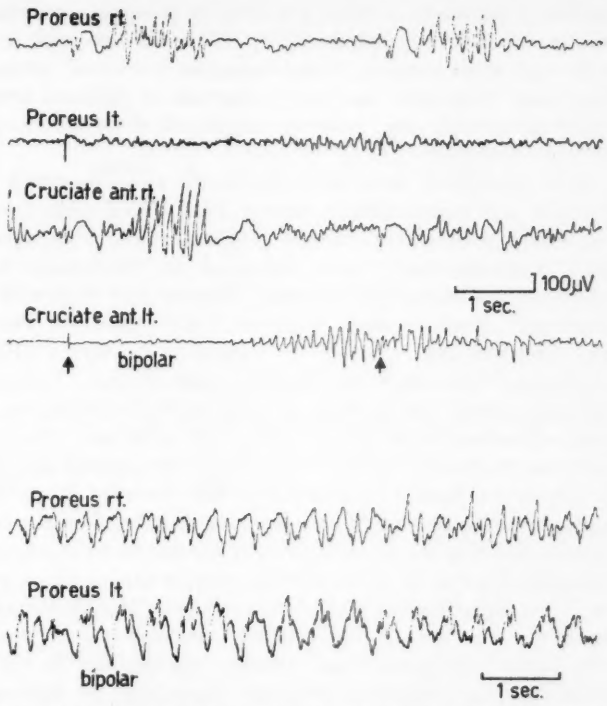

Fig. 4 A. Cortical responses, with homolateral predominance, elicited by stimulation of the *right* gyrus cinguli at frontal plane + 7.5 with single shocks (indicated by arrows) of 20 V and 2 msec. duration. Note "spike *and* slow wave" appearance of homolateral response which is followed by repetitive afterdischarge of "spindle" type (cf. fig. 3). Nembutal preparation.

Fig. 4 B. Spike and wave responses in frontal parts of the cortex from stimulating the hippocampus at frontal plane + 2 (lateral rt. 8 to 9) with 2.5 pulses per second of 15 V and 1.1 msec. duration. *Encéphale isolé* preparation with nembutal.

1950). Responses elicited from the gyrus cinguli (fig. 4 A) were most pronounced in the homolateral hemisphere. Latency studies with the oscilloscope demonstrated that the spike and wave responses — when present — had a varying latency, thus not always of the length met with in most thalamic recruiting potentials (JASPER 1949 a; HANBERY, AJMONE-MARSAN and DILWORTH 1954). In other words, under suitable conditions spike and wave responses may be elicited in the cortex through more direct ("specific") as well as through polysynaptic ("un-

specific") pathways, a finding which, in principle, confirms the results of HUNTER and INGVAR (1955) with metrazol induced spike and wave activity. Some variations in cortical distribution were seen with spike and wave responses of different latencies. These differences were, however, vague and never permitted any definite conclusions.

The subcortical structures stimulated also comprised diencephalic and mesencephalic regions from which other forms of bilaterally wide-spread cortical responses have been reported. Such responses have been described as "secondary sensory responses" (DERBYSHIRE, REMPEL, FORBES and LAMBERT 1936, DEMPSEY, MORISON and MORISON 1941; MORISON, DEMPSEY and MORISON 1941 a and b) or "secondary discharges" (FORBES, BATTISTA, CHATFIELD and GARCIA 1949; STARZL, TAYLOR and MAGOUN 1951). Apart from a wide cortical distribution, they were characterized by a long latency (30 to 80 msec.) and by the fact that they did not follow stimulation frequencies higher than 5 per second. Similar responses were also observed in the present series which diminished in amplitude with increasing frequency of stimulation (fig. 5). It is at present unclear to what extent such responses are related to those which may be driven into a pattern of 3 per second spike and wave potentials. Double stimulation, with one electrode in the thalamic reticular system (producing the "spike" component) and another one caudally in the brain stem (in the tegmental reticular formation, in attempts to produce the "slow wave" component), consistently failed to reproduce the typical spike and wave pattern.

During a similar stimulation with two electrodes, an observation was made which was later confirmed in several preparations. High frequency (200 c/s) electrical stimulation of the tegmental reticular formation, "activating" the cortex (MORUZZI and MAGOUN 1949), changed the form of the cortical spike response, elicited from the anterior (thalamic) electrode, into a spike *and wave* response. This, however, afterwards slowly returned to the original spike form (fig. 6; see Discussion).

The subcortical structures yielding cortical spike and wave responses on electrical stimulation are shown in fig. 7. The dotted areas indicate those from which responses were obtained only with higher stimulation intensities. The darker (striped) area indicates structures from which spike and wave (or slow wave) responses were obtained more constantly or with lower stimulus

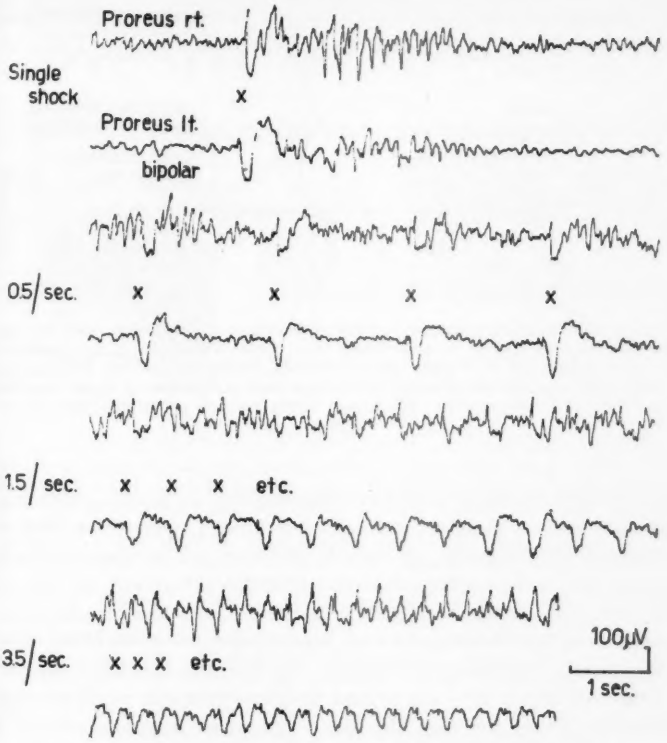

Fig. 5. Bilateral frontal slow wave responses elicited by stimulation of the mesencephalic reticular formation at frontal plane + 2. (12 V, 2 msec. duration of pulses.) Note change in amplitude with increasing frequency of stimulation. Nembutal preparation.

intensities. In general, within the striped area, the responses elicited rostrally were more often of the spike variant, those from caudal regions more slow-wave like. The latter were usually more symmetrical, a fact, which would seem to be consistent with existing knowledge of connections between the two halves of the brain stem in the areas in question (WALKER 1938).

Discussion.

The data have indicated that cortical spike and wave responses — driven by a 3 per second stimulation so as to resemble electro-

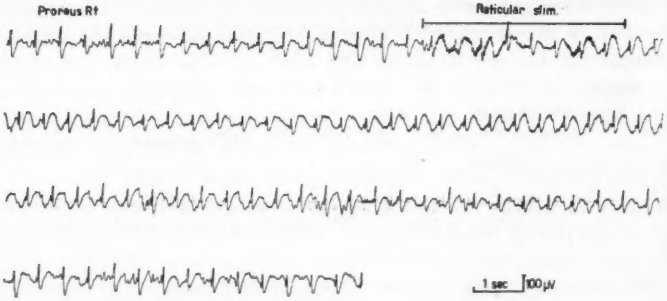

Fig. 6. Effect of high frequency (200 c/s) reticular stimulation upon frontal cortical response elicited by stimulation of n. centralis medialis of the thalamus (2 per second, 18 V, 1 msec. pulse duration). Continuous record. Preparation under light nembutal anesthesia. Note change from predominantly spike response into spike *and wave* response which then slowly assumes its original spike shape again.

graphically the pattern of petit mal epilepsy in man — were not elicited from a precisely definable, or known, anatomical entity situated subcortically. It should, however, be emphasized that structures, assigned to the thalamic reticular system, as well as to the mesencephalic reticular formation, appeared to be those from which cortical spike and wave responses most often were produced by electrical stimulation. The results have demonstrated many discrepancies — in regard to form, symmetry and cortical distribution — between the spike and wave responses obtained in the experiments presented and the classical EEG pattern of petit mal epilepsy in man. The author is therefore of the opinion that his data cannot be adduced in support of the theory of a subcortical pace-maker mechanism for the 3 per second spike and wave epilepsy, *i. e.*, a pace-maker in the brain stem which drives a *passive* cortex (PENFIELD and JASPER 1954, p. 482). This view is further supported by the findings of COHN (1949) and of KOPELOFF, WHITTIER, PACELLA and KOPELOFF (1950) who failed to reproduce the typical 3 per second spike and wave pattern by strychninizing medial thalamic structures (in cats) and by trying deposits of epileptogenous alumina cream in the same region (in monkeys) respectively. The support of these investigations is, however, limited by the fact that central neurons may be insensitive to chemical stimulation (cf. FRANKENHAEUSER 1951).

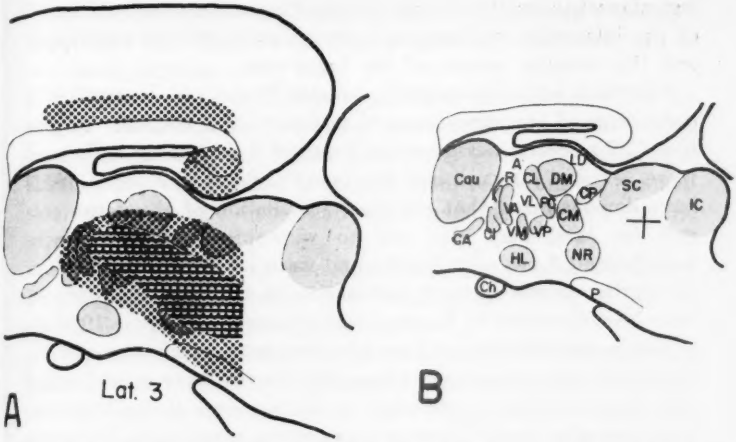

Fig. 7 A. Diagram summarizing stimulation points from which (frontal) cortical responses of the spike, the slow wave or the spike and wave type were produced. Stippled areas: higher stimulation intensity required for typical cortical responses; striped area: lower stimulation intensity required. Cortical responses obtained from stimulation of the gyrus cinguli were predominantly homolateral (Fig. 4 A). The diagram represents a parasagittal section through the cat's brain 3 millimeters from the midline.

Fig. 7 B. Principal structures of the brain stem in the plane of fig. 7 A. A anterior nuclei of the thalamus; CA anterior commissure; Cau n. caudatus; Ch chiasma; CI internal capsule; CL n. centralis lateralis; CM n. centrum medianum; CP posterior commissure; DM n. medialis dorsalis; HL lateral hypothalamus; IC inferior colliculus; LD n. lateralis dorsalis; NR n. ruber; P cerebral peduncle; PC n. paracentralis; SC superior colliculus; VA n. ventralis anterior; VL n. ventralis lateralis; VM n. ventralis medialis; VP n. ventralis posterior. The cross indicates frontal and horizontal stereotaxic planes zero.

The view presented does not, however, amount to a denial of the rôle of the thalamic reticular system for cortical electrical activity, so well established by JASPER and his collaborators and by others (see references above). It has often been stressed by JASPER and by PENFIELD (see PENFIELD and JASPER 1954) that this system, or the entire reticular system, is, in fact, the only known functional entity of the brain, capable of influencing the cortex in the wide-spread way which is required to explain the generalized abnormality of the EEG during a petit mal seizure. Clinical studies have clearly established the disturbances of consciousness which accompany petit mal seizures. As has been pointed out by PENFIELD and JASPER (1947, 1954), this evidence also strongly suggests a relationship between the pathophysio-

logical mechanism of petit mal epilepsy ("centrencephalic epilepsy" of the bilaterally synchronous 3 per second spike and wave type) and the reticular system of the brain stem.

PENFIELD and JASPER (1947; see also TÜKEL and JASPER 1952) early stressed the importance of medial frontal cortical "trigger zones" for certain asymmetrical forms of 3 per second spike and wave activity. LENNOX and ROBINSON (1951) and KAADA (1951) have demonstrated that electrical stimulation of the gyrus cinguli (in cats) may give rise to wide-spread (predominantly homolateral) 3 per second spike and wave activity. Other factors in relation to the cortical distribution of the petit mal pattern have been discussed by KAADA (1952). Elsewhere (INGVAR 1955 a), experimental observations have been presented which demonstrate that neuronal mechanisms, responsible for the 3 per second spike and wave activity proper, may be entirely cortical. Such potentials may also spread through transcortical connections (HUNTER and INGVAR 1955). This evidence, suggesting *active* cortical mechanisms responsible for the typical petit mal pattern (cf. GIBBS and GIBBS 1952), will not exclude a primary disturbance of brain stem mechanisms in this disease.

Many problems are still unclarified concerning the cortical conditions necessary for the 3 per second spike and wave activity, conditions, which in the present experiments have eluded control. The enhancement of cortical spike and wave potentials (fig. 6) as well as the opposite, a suppression (MORUZZI and MAGOUN 1949; ARDUINI and MORUZZI 1953), by electrical stimulation of the reticular activating system indicates that this system may, to some extent at least, determine the conditions referred to. It remains, however, a matter of speculation whether extraneuronal influences upon the cortex from the reticular system (INGVAR 1955 b) have any significance for the development of bilateral synchronous 3 per second spike and wave activity in petit mal epilepsy.

Summary.

1. Cortical 3 per second spike and wave activity — resembling the typical EEG potentials of petit mal in man — has been elicited by subcortical electrical stimulation in cats. On stimulation of medial thalamic structures the results in many respects confirm those of JASPER and DROOGLEEVER-FORTUYN (1947).

2. The conditions for obtaining cortical "recruiting responses" (DEMPSEY and MORISON 1942) and spike and wave responses do not seem identical.

3. It has not been possible to establish the existence of a definable anatomical or functional anatomical subcortical system — consistent with any known such entity — which, on electrical stimulation, will produce a specific form of bilaterally symmetrical 3 per second spike and wave potentials with a general cortical distribution.

4. The results are not consistent with the pace-maker theory which presumes a brain stem mechanism driving a *passive* cortex during the petit mal seizure.

5. It is emphasized that the reticular system of the brain stem still appears to be the only functional entity known, capable of the wide-spread influence upon the cortex which is required to explain the generalized disturbance of the EEG during a petit mal seizure.

The author wants to acknowledge the unfailing help and guidance obtained from Dr. HERBERT JASPER during the course of this study. The author was supported by a grant from the Swedish Medical Research Council.

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Electrical Activity of Isolated Cortex in the Unanesthetized Cat with Intact Brain Stem.

By

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It is an established fact that the cerebral cortex, partially or completely isolated from other neuronal structures, possesses the capacity of generating rhythmic electrical activity (BREMER 1935, 1937, 1949; JASPER 1949 b, 1949 c; KRISTIANSEN and COURTOIS 1949; BURNS 1950, 1951; ECHLIN, ARNETT and ZOLL 1952; HENRY and SCOVILLE 1952; WRIGHT, ANDREW and JACOBSON 1954). For the present investigation the technique of the completely isolated cortex was chosen in order to investigate whether such cortex would be able to produce 3 per second spike and wave activity independent of subcortical influences. Clinical as well as experimental observations (see Discussion) have suggested that such rhythms, resembling the EEG pattern of centrencephalic epilepsy of the petit mal type, may be produced by the cortex itself without driving from subcortical structures.

With the technique employed it was observed that the isolated area became spontaneously active, producing an episodic "burst" type of activity similar to what has been described in completely

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A preliminary report of this investigation was presented at the III International Congress of EEG in Boston 1953 (INGVAR 1953).

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isolated cortex by other workers (KRISTIANSEN and COURTOIS 1949; BURNS 1950, 1951; BURNS and GRAFSTEIN 1952; ECHLIN, ARNETT and ZOLL 1952; HENRY and SCOVILLE 1952). In these episodes very often a dominant component of 2.5 to 3.5 per second "synchronous" slow waves of a high amplitude could be distinguished. On several occasions a regular 2.5 to 3.5 per second spike and wave activity was obtained from the isolated area, both spontaneously as well as on chemical stimulation. It was furthermore observed that the electrical activity of the isolated area could be influenced by electrical stimulation of the ascending reticular formation of the brain stem. The latter finding will be dealt with in a separate communication (INGVAR 1955).

Technique.

Twenty-seven adult cats were used. They were prepared under intravenous pentothal anesthesia initiated under ether. Artificial respiration through a tracheal cannula was used from the start of the experiment. When the operative procedures had been performed, the cat's head was placed in a stereotaxic instrument and local anesthesia (Nupercain) applied around the exposure. The pentothal anesthesia was then discontinued and the animal immobilized with curare. The animal was kept at a temperature of 38° to 39° C measured rectally. In some experiments a small dose of Coramine was given intravenously to improve circulatory conditions. During the following hour or two, all effects of the barbiturate disappeared in the EEG, and lively reflexes could be demonstrated if the curare concentration were allowed to fall. The experiment was thus performed in an unanesthetized preparation with intact brain stem.

Both hemispheres were exposed widely. The isolation was carried out by subpial dissection in one hemisphere with a small spatula, according to the technique of KRISTIANSEN and COURTOIS (1949) and BURNS (1950, 1951). The isolated cortical slab comprised in all experiments a rather large area of the *parietal* cortex, measuring in greatest length and width about 30 × 20 mm and in depth in the middle about 10 mm. It comprised one half of the lateral gyrus and one half of the ectosylvian gyrus with the intact middle suprasylvian gyrus in between. It reached from the posterior edge of the hemisphere to the posterior cruciate gyrus. The reason for choosing a slab of this size was twofold. It was found that with the technique of recording used, difficulties were encountered with small slabs (performed on one gyrus) in trying to avoid effects of pick-up from the activity of the surrounding intact cortex. Even if such a small slab was killed by freezing or destruction of its circulation, an activity very similar to that of the surroundings could be recorded over it but with a reduced amplitude. This was not found to be the case with a slab of the size used. Furthermore, a large

slab was chosen to include a larger mass of cortical elements and some longer transcortical connections of the white matter in the isolated area.

As soon as the isolation had been performed, a paraffin pool (the paraffin kept at 38° C) was arranged over the cortex by attaching the skin to a metal ring (BURNS 1950).

The electrodes used for recording from the isolated and intact cortex consisted of fine flexible silver or platinum wires, often bent in a spiral and fused into small spheres at their ends. In several experiments fine cotton wicks soaked in saline were wrapped around the metal wires and used as electrodes in order to eliminate completely any mechanical trauma to the cortical surface during the recording.

Confirming BURNS (1950), it was found inadvisable to use "monopolar" leads (indifferent electrode on bone or nose) in recording from the slab owing to pick-up effects from intact cortex. 'Bipolar' leads in single pairs or chains with an inter-electrode distance of 3 mm was therefore employed throughout the study. They were placed over the central parts of the slab. The potentials recorded were fed to an Offner Dynograph or a Grass Model 3 EEG machine which was usually used with its largest time constant (0.5 seconds, other time constants indicated in the illustrations). Films from a multiple beam cathode ray oscilloscope (no distortion of frequencies up to 10,000 c/s) were also obtained.

The isolation was checked in most experiments by one or several of the following methods. Neuronal discontinuity between the isolated area and the intact surrounding cortex as well as the subcortical structures was demonstrated by evoked potential techniques (visual, auditory, somato-sensory stimulation) and by electrical stimulation of specific and unspecific thalamic nuclei with control leads in the intact hemisphere. Metrazol or strychnine spikes were induced inside or outside the slab. These measures, as well as electrical stimulation with after discharges, all failed to demonstrate any neuronal connections between the isolated area and the surroundings. In 18 of the experiments, furthermore, serial paraffin sections were obtained of the hemisphere in which the isolation had been performed. In all cases the histological evidence demonstrated that the subpial dissection had yielded a complete neuronal isolation.

Chemical stimulation was carried out by placing small squares (3 × 3 mm) of filter paper on the cortical surface after soaking them in a solution of the stimulating agent (usually 10 % metrazol, in a few experiments 3 % strychnine).

Results.

Electrical activity was recorded from the isolated area of parietal cortex in all except four experiments. In these four cases neither chemical nor electrical stimulation could produce any activity in the slab.

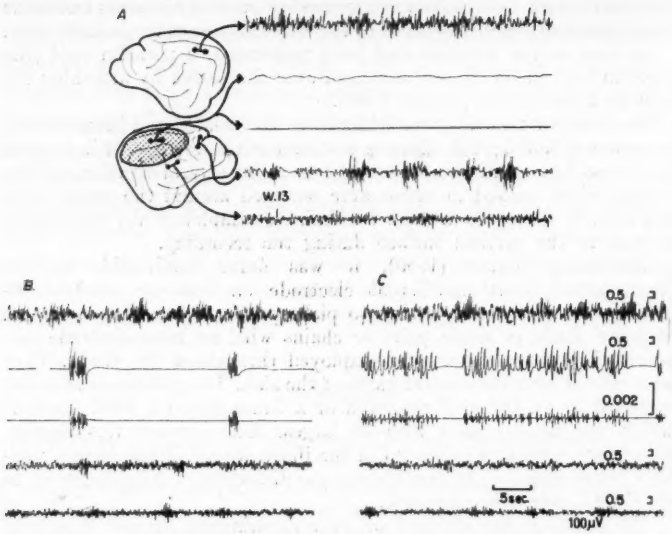

Fig. 1. Appearance of spontaneous activity in isolated cortex during recovery from anesthesia. Interval between 1 A and 1 B 1 hour, between 1 B and 1 C 20 minutes. Recording from isolated cortex with one pair of electrodes and two channels with long and short time constants (0.5 and 0.002 seconds) as indicated.

Preliminary observations indicated that with the preparations used, electrical activity tended to develop spontaneously in the slab. In several experiments the spontaneity of the activity was especially studied by starting the recording while signs of the barbiturate anesthesia (so called barbiturate spindles of 8 to 12 per second frequency) still persisted in the intact cortex. During this period the slab was electrically silent (Fig. 1 A). When the typical activity of the unanesthetized cortex (fast frequencies of a lower voltage with a greatly reduced or absent spindling) slowly developed in the intact surroundings following some hours, electrical activity in the form of paroxysmal episodes ("bursts", see below) began to develop in the slab in all the experiments (Fig. 1 B). The episodes then became more frequent (Fig. 1 C) and in some experiments an almost continuous activity was recorded from the isolated area (Fig. 1 C and 3 C). As a rule, the average amplitude of the slab activity (150 to 800 μV) became somewhat reduced the more continuous the activity was. It was only rarely lower than that of the intact surrounding (50 to 500 μV) with

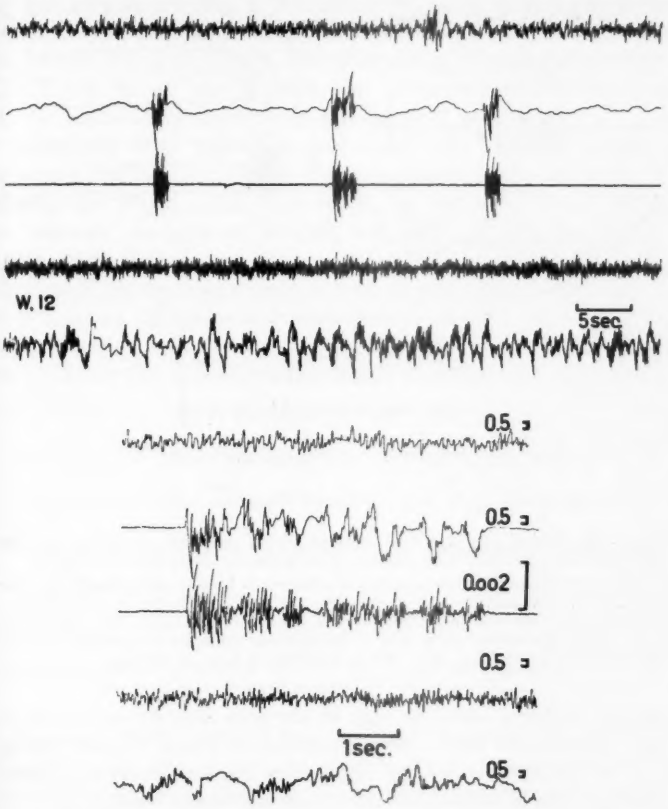

Fig. 2. Spontaneous "bursts" from isolated cortex recorded with slow and fast paper speed. Recording as in fig. 1. Cal. 100 μV.

identical recording technique. It was found that the slab activity, once it developed after the initial anesthesia, could again be reversibly blocked by intravenous pentothal or nembutal, a finding which confirms the observations by BURNS (1951).

The spontaneous episodic bursts usually lasted from 1 to 8 seconds and consisted of both fast and slow frequencies (Fig. 1, 2, 3 and 5). It should be emphasized that most frequencies and wave forms met with in the activity from intact cortex were also encountered in the slab, but in a very irregular form. Only a few

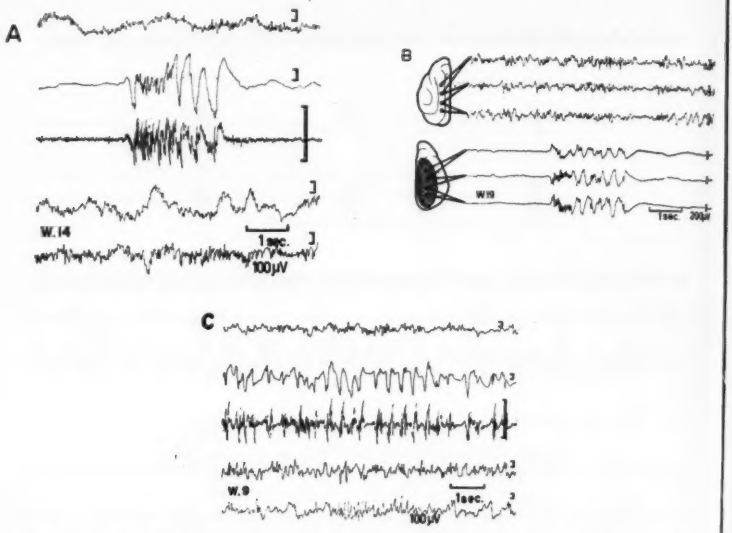

Fig. 3. A. Typical episodes from isolated cortex initiated by fast frequencies which are later followed by 3 per second slow waves. Recording as in fig. 1.
B. The same phenomenon in another experiment in bipolar chain recording. Time constant 0.5 seconds.
C. Continuous activity in isolated cortex demonstrating trains of 3 per second slow waves. Recording as in fig. 1. In all three examples the relation between the fast activity and the slow waves, described in text, is evident.

regular features could be seen in the slab activity in a more or less pronounced form. They consisted in trains of low voltage (10 to 100 microvolts) *fast frequencies* and *slow waves* of a higher voltage (150 to 800 microvolts) from the "delta" band (0.5 to 3.5 per second frequencies) with a marked dominance of 2.5 to 3.5 per second frequencies. For the sake of brevity the slow wave forms will be termed "3 per second rhythms" in the following.

In oscilloscopic studies the fast waves were found to consist of frequencies of up to 55 to 65 cycles per second. It seems reasonable to assume that this activity is identical with the one (low voltage 60 to 70 per second frequencies) described by BURNS (1951) which takes part in the repetitive discharges evoked on electrical stimulation of isolated cortex in *cerveau isolé* preparations.

Three per second rhythms were found in a varying degree in all experiments in which activity developed in the slab. In an episode it was usually observed that the slab activity initially consisted

of fast frequencies (Fig. 2, 3 A and 3 B). Later, after a second or so, it frequently changed into the high voltage slow waves of 2.5 to 3.5 per second frequency. The alternation between fast activity and 3 per second waves could also appear repeatedly during a longer episode. An example of this is given in fig. 3 C.

Recording with high gain and short time constant demonstrated a marked suppression of the amplitude of the fast activity during the height of the slow waves, the fast activity appearing in "showers" in between the slow waves with higher amplitudes. Often these 3 per second "showers" of fast activity were "synchronized" in their turn into a few, or one single larger spike, thus creating the spike and wave pattern. A well formed 2.5 to 3.5 per second spike and wave activity (Fig. 4) developed spontaneously in the slab in 4 experiments and was seen transiently in 8 others.

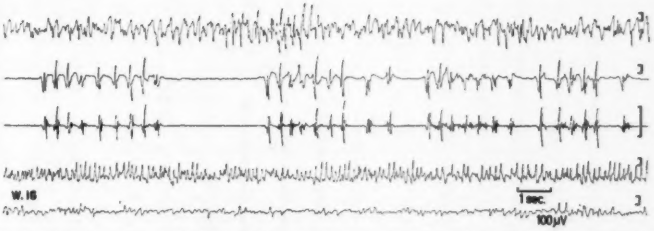

Fig. 4. Spontaneous episodes of 2.5 to 3 per second spike and wave activity recorded from isolated cortex. Recording as in fig. 1.

Recording with multiple leads (bipolar chain of 4 electrodes) along the slab surface with an inter-electrode distance of 3 mm clearly demonstrated that the 3 per second rhythms, in contrast to the fast frequencies, appear in synchrony in the EEG record simultaneously in all parts of the slab with phase reversals (Fig. 3 B and 5).

As is evident from the illustrations slow waves of other frequencies were also recorded in the slab. They were usually very irregular and seldom showed a definite relation to the fast waves. Some of them are probably short "silent periods". They may also, of course, represent slow potential phenomena deformed by the limited frequency response of the recording equipment.

Confirming the findings of SINGH, AMADOR, SENCER and CRANDALL (1951) and HUNTER and INGVAR (1955), it was found that metrazol enhances the development of 3 per second activity in

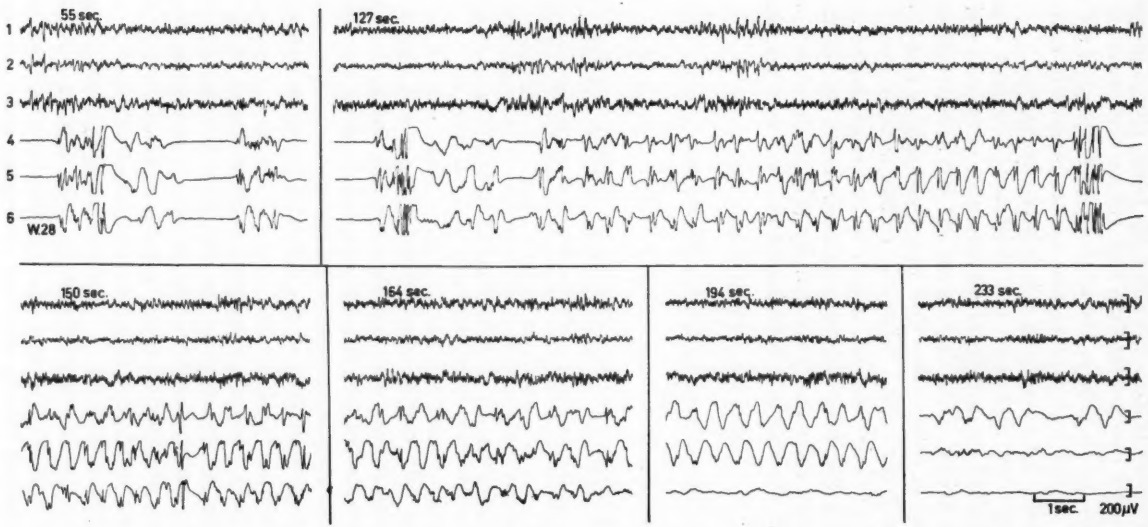

Fig. 5. Chemically induced 2.5 to 3 per second spike and wave activity in isolated cortex. Channels 1, 2 and 3 bipolar chain recording from intact cortex. Channels 4, 5 and 6 same from isolated cortex, as in fig. 3 B. Metrazol was applied between electrodes recording in channel 6, 55 seconds before first sample. See text. Time constant 0.5 seconds.

the cortex even in the absence of subcortical connections. In fig. 5 an example is given of such activity, produced in the isolated cortex by metrazol, locally applied to the cortical surface. The typical pattern first developed around the two most occipital electrodes where the small squares of filter paper were placed. The 3 per second spike and wave activity then included the whole slab. It was occasionally interrupted by paroxysms of high voltage fast waves (distorted by the pens) which were often followed by short "postparoxysmal" silent periods. As was often the case on chemical stimulation, a depression of the electrical activity developed at the site originally stimulated. This depression showed many similarities to the "spreading depression" of LEÃO (1944) in its slow spread and slow recovery over many minutes. It is of interest to note that the character of the electrographic pattern in areas around the complete depression changed from a spike and wave type to a slow wave type of 3 per second. The depression in this case finally involved the whole slab. Some conditions necessary for the appearance of 3 per second rhythms will be discussed in the following.

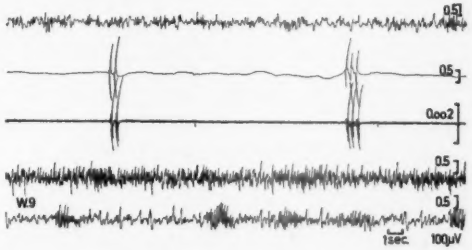

Fig. 6. 3 per second spikes from isolated cortex. Recording as in fig. 1.

If local stimulation of the slab with metrazol (or strychnine) was maintained, large spikes were occasionally produced which, at first, repeated themselves at a 3 per second rate with a less pronounced or inconspicuous slow wave in between. High voltage 3 per second spikes were also observed to occur spontaneously, initially when the slab started to become active after the anesthesia (Fig. 6). In the metrazol experiments the 3 per second spikes usually became more frequent in a paroxysmal fashion and a complete "epileptic seizure" of varying length might ensue with a "tonic" and a "clonic" phase. Such epileptic seizures remained

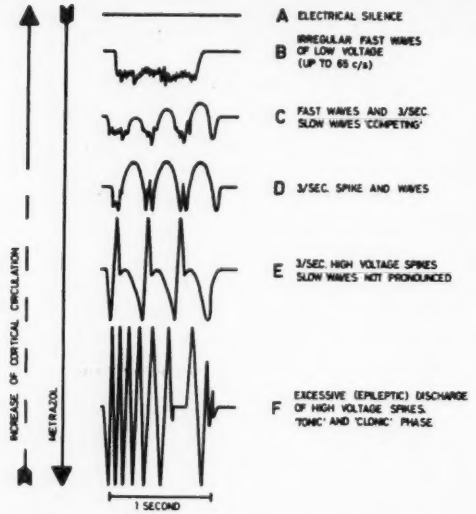
A ELECTRICAL SILENCE
B IRREGULAR FAST WAVES OF LOW VOLTAGE (UP TO 65 c/s)
C FAST WAVES AND 3/SEC. SLOW WAVES 'COMPETING'
D 3/SEC. SPIKE AND WAVES
E 3/SEC. HIGH VOLTAGE SPIKES SLOW WAVES NOT PRONOUNCED
F EXCESSIVE (EPILEPTIC) DISCHARGE OF HIGH VOLTAGE SPIKES. 'TONIC' AND 'CLONIC' PHASE
INCREASE OF CORTICAL CIRCULATION
METRAZOL
1 SECOND

Fig. 7. Hypothetical interrelation between various forms of spontaneous and chemically induced electrical activity of isolated cortex. See text.

localized to the slab. As has been pointed out by KRISTIANSEN and COURTOIS (1949), they cannot be distinguished electrographically from epileptic manifestations in intact cortex. Illustrations of epileptic seizures in isolated cortex are given elsewhere (INGVAR 1955).

Discussion.

Any discussion of the results presented and those of other studies of isolated cortex should recognize the fact that important differences in technique are present. Results from a large area in the parietal cortex of the cat may only in a general way be compared with those from other species using isolated areas of different sizes in other cortical regions. There are no data available as to the importance of cyto-architectonic differences for the electrical activity of an isolated cortical area. The importance of its size would also require a more complete investigation from what has been indicated by the observations of BURNS (1950).

Nevertheless, the conclusion seems justified that the electrical activity of completely isolated cortex of various species under

unanesthetized conditions is characterized by episodic, paroxysmal, usually irregular "bursts" containing fast frequencies of a lower voltage and slow waves of a higher voltage. Such activity has been described by KRISTIANSEN and COURTOIS (1949) and BURNS (1950, 1951) in isolated cortex in the cat, by ECHLIN, ARNETT and ZOLL (1952) in cat, monkey and man and by HENRY and SCOVILLE (1952) in man.

The results presented as well as the observations by others indicate a rather great quantitative variability in the activity recorded from isolated cortex. The vascularization of the slab, ultimately depending upon the subpial dissection technique, probably to some extent explains variations in the amount of activity produced by the isolated area under "normal" as well as "epileptic" conditions.

The spontaneous occurrence of electrical activity in the isolated area.

It was found that electrical activity appeared spontaneously in the isolated cortical area. This confirms the investigations of ECHLIN, ARNETT and ZOLL (1952) and HENRY and SCOVILLE (1952). Both groups studied isolated cortex under conditions in principle similar to those of the present series, *i. e.*, under unanesthetized conditions in brains with intact brain stem. KRISTIANSEN and COURTOIS (1949) also reported spontaneous electrical activity in isolated cortex in nembutalized cats or *cerveau isolé* preparations (see below). BURNS (1950, 1951), on the other hand, states that isolated cortical areas do not become electrically active unless stimulated from an outside source. Such was the case in the great majority of experiments which were carried out in *cerveau isolé* preparations or modifications thereof. Once started by electrical stimulation, however, the activity of the isolated slab would go on for many minutes with a burst type of activity which then would disappear, and the slab become silent again. As mentioned already, in spite of differences in recording technique, there seem to be reasons to identify the activity recorded by BURNS with the one reported here.

The investigations quoted indicate that spontaneous activity develops in isolated cortex if the brain stem is intact, but that this is usually not the case if this structure has been interfered with. Apparently, the excitability of the slab which may vary

considerably (BURNS 1950, 1951), is higher in preparations with an intact brain stem than in those of the *cerveau isolé* type. In other words, there would be a decrease of excitability of the slab concomitant with the decrease that is taking place in the excitability of intact cortex, when brain stem structures are interrupted at the midbrain level (BREMER 1935, 1937; LINDSLEY, BOWDEN and MAGOUN 1949). This view implies that the isolated part of the cortex would be subjected to the influence of extraneuronal factors which in some way are related to the brain stem.

Elsewhere (INGVAR 1953, 1955) the experimental evidence has been given for the view that extraneuronal mechanisms play a rôle in the regulation of the cortical excitability state, and that these mechanisms, to some extent at least, are regulated from the brain stem. It was observed that high frequency electrical stimulation of the brain stem reticular system may be accompanied by an increase in the cortical circulation and that, following this increase, electrical activity may start in a previously electrically silent and neuronally completely isolated slab. In view of these findings it seems reasonable to assume that extraneuronal events (acting through circulatory and/or humoral factors) might explain the appearance of "spontaneous" electrical activity in isolated cerebral cortex in the preparations used (see BREMER 1953).

The form of activity of the isolated area.

As mentioned, other workers have described the low voltage fast and the high voltage slow activity recorded from isolated cortex. The 3 per second character which has been such a striking observation in the present series, has not been noted previously. It is of interest, however, that in experiments with similar technique, 3 per second slow waves actually have been recorded (*e. g.*, ECHLIN, ARNETT and ZOLL 1952, Fig. 6). BURNS (1951) and BURNS and GRAFSTEIN (1952) generally studied smaller slabs and they usually analyzed only the initial phase of the bursts from isolated cortex during which the 3 per second waves are less likely to appear.

KRISTIANSEN and COURTOIS (1949) reported the spontaneous occurrence of an activity "closely related to the alpha rhythm" in isolated parietal cortex of the cat. The amplitude of this activity was around 25 % of that of a similar activity recorded from the

surroundings. With the small isolated area used by these workers it would seem that pick-up from intact surroundings might have been of importance for such a low voltage activity. It is of interest, though, that these workers were able to produce very regular 10 per second high voltage activity by local treatment of the isolated cortex with eserine and acetylcholine. HENRY and SCOVILLE (1952), using larger slabs, have pointed out some similarities between barbiturate spindles and the slab activity. In the experiments presented it has not been the impression that "alpha activity" (defined as regular 8 to 12 per second frequencies in a more or less pronounced spindle form) is a characteristic component of the slab activity. Apart from the few features described, it was found that the activity of the isolated area, in contrast to that of the intact surroundings, was characterized by a *lack of regular patterns* of the frequencies between the very fast and the very slow.

The data, therefore, do not justify the generalization that the production of the alpha rhythm as such is an entirely cortical phenomenon, since there is such a marked difference between this form of activity and the one which the *completely* isolated slab is capable of producing. The results are, in fact, not inconsistent with the view that the regulation of the alpha rhythm, and most other *regular* cortical rhythms, is based upon some form of interaction between "semi-independent" electrically active cortical and subcortical neuronal aggregates (DUSSER DE BARENNE and MCCULLOCH 1941; MORISON and DEMPSEY 1942; MORISON, FINLEY and LOTHROP 1943; CHANG 1950; see also JASPER 1949 b).

ECHLIN, ARNETT and ZOLL (1952) have evoked CANNON's "law of denervation" in order to explain the paroxysmal nature of the discharges ("suppression bursts" (SWANK and WATSON 1949)) from isolated cortex. They suggest that "sensitization" by partial denervation may be a factor involved in focal cortical epilepsy. HENRY and SCOVILLE (1952), discussing their findings in isolated cortex in man, suggest that the release of the isolated cortex from subcortical control may be held responsible for the character of the activity recorded from the slab.

The suggestions quoted, as well as many theoretical contributions to the origin of cortical electrical rhythms, have, it seems, mostly considered purely interneuronal relationships. A secondary rôle has usually been attributed to other factors, *e. g.*, to those of the *milieu interne*, as determinants of the cortical

electrical activity. Since it has been demonstrated that cortical circulatory conditions may be correlated with the EEG, in isolated cortex as well (INGVAR 1953, 1955), it would, therefore, seem premature to explain the characteristics of the electrical activity of isolated cortex as release phenomena chiefly on the basis of interruption of neuronal connections to it. Increased knowledge particularly of circulatory conditions in isolated cortex is required before the features of its electrical activity can be understood and related to findings in intact cortex.

The 3 per second spike and wave activity.

The episodes of 3 per second spike and wave activity recorded from isolated cortex merit special emphasis since they have an obvious interest for the question of the origin of similar rhythms in petit mal epilepsy (see GIBBS and GIBBS 1952 and PENFIELD and JASPER 1954).

The importance of cortical mechanisms for the production of 3 per second spike and wave patterns has been stressed previously by PENFIELD and JASPER (1947) and JASPER (1949 a and c) in clinical studies. The cortical origin of these rhythms has also been indicated in studies by HAYNE, BELINSON and GIBBS (1949) and JUNG and RIECHERT (1952) in deep recordings in humans suffering from petit mal epilepsy. The evidence of these two studies is, however, contradicted, by that of WILLIAMS (1953). The experimental studies of SINGH, AMADOR, SENCER and CRANDALL (1951) and of SHIMIZU, REFSUM and GIBBS (1952) indicate that 3 per second spike and wave patterns may be produced with metrazol in the cortex of the cat independently of subcortical structures. HUNTER and INGVAR (1955) have demonstrated a 3 per second character of the cortical transmission of so called irradiated visual responses under metrazol. WRIGHT, ANDREW and JACOBSON (1954), in a recent study, also observed 3 per second rhythmic phenomena in isolated cortex in nembutalized cats, after strychninization and electrical stimulation. In the experiments presented here, it has been clearly demonstrated that the cortex itself — in the cat at least — possesses the mechanisms necessary for generating the 3 per second spike and wave pattern. There is thus rather abundant experimental and some clinical evidence that this electrographic pattern may originate in the cortex.

It was emphasized above that a certain cortical state is necessary for the spike and wave pattern in isolated cortex, a state which may vary and thus change the general character of the pattern from predominantly 3 per second spike and waves to predominantly 3 per second waves. Although the exact nature of this cortical state remains obscure, a few points might be brought up for discussion.

In the diagram of fig. 7 the various forms of activity observed in isolated cortex have been hypothetically correlated. The relations have been indicated between the fast activity, the 3 per second rhythms and the high voltage, high frequency epileptic discharges. Metrazol (or strychnine) enhances the cortical patterns as indicated by the arrow, but stages A, B, C, D and E have all been recorded from the slab without chemical interference. The other arrow in the diagram indicates the effects of an increase of the cortical circulation observed after electrical stimulation of the reticular system (INGVAR 1955). The effect upon the spontaneous activity (usually stage C) was often that of an increase of fast activity. The influence of cortical circulatory changes upon epileptic discharges was less clear but generally tended to result in a desynchronization, *i. e.* a change of pattern from high voltage potentials of a comparatively low frequency to a pattern of faster frequencies of lower voltage. However, the complete change from stages F to B was never observed. After an epileptic seizure or long lasting periods of 3 per second rhythms the activity of the slab usually remained atypical and sometimes depressed. This indicates that not only circulatory but also other factors may influence the electrical events in isolated cortex. Probably ionic conditions are of great importance (GERARD 1938). BURNS and GRAFSTEIN (1953) have clearly demonstrated the rôle played by potassium in depression states of isolated cortex. The influence of the ionic environment for the frequency of discharges of isolated nervous structures in amphibians has also been pointed out recently by DESMEDT (1954).

It is evident that much knowledge is needed to explain why 3 per second spike and wave activity sometimes does appear and sometimes does not in isolated cortex. Until such knowledge is at hand it is not possible to establish the functional significance of these rhythms. It should be emphasized that the data presented do not contribute to an explanation of other very important features of the typical petit mal epilepsy discharges in

man, such as the bilateral sudden onset, the cortical distribution of the typical pattern and the relation of the pattern to other normal cortical rhythms. It would therefore seem too early to correlate the findings in isolated cortex with the theory of a subcortical pace-maker mechanism proposed for centrencephalic epilepsy of this type (JASPER and DROOGLEEVER-FORTUYN 1947; PENFIELD and JASPER 1947, 1954).

Summary.

1. The electrical activity of isolated parietal cortex in the unanesthetized cat with intact brain stem has been analyzed. The data confirm that neuronally completely isolated cortex with preserved blood supply has the capacity of generating rhythmic electrical activity.

2. Electrical activity appeared spontaneously in the isolated areas, a fact confirming other studies with similar preparations. The spontaneous activity could be reversibly blocked by small doses of barbiturates. Brain stem mechanisms which were intact may have increased the excitability of the isolated area through extraneuronal means. The excitable slab becomes electrically active — it is assumed — due to the 'stimulating' influence of circulatory and/or humoral factors in the cortex.

3. The paroxysmal bursts, recorded from isolated cortex, showed a strong resemblance to a similar activity reported by other workers. A slow wave component of the bursts was found to be dominated by 2.5 to 3.5 per second waves of high voltage which often attained very regular characteristics. 3 per second sinusoidal forms as well as 3 per second spike and wave forms were often recorded. In the EEG record these rhythms appeared synchronous over the isolated area. The data demonstrate that the cortex itself possesses the inherent mechanisms necessary for the production of a 3 per second pattern similar to the one met with in petit mal epilepsy. Some conditions for the appearance of such a pattern in isolated cortex are discussed.

4. Apart from the 3 per second rhythms and a fast frequency component (of frequencies of up to 65 c/s), the slab activity was highly irregular, demonstrating a variety of frequencies and wave forms seen in the routine EEG. The data seem consistent with the view that the regulation and maintenance of the alpha rhythm, as well as other *regular* cortical patterns, are based upon an inter-

action between the cortex and other neuronal structures (cortical as well as subcortical).

5. It is emphasized that not only purely interneuronal relationships, but also circulatory conditions as well as other factors — as yet unknown — may determine the form and frequency of rhythmic electrical events of the cortex.

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Extraneuronal Influences Upon the Electrical Activity of Isolated Cortex Following Stimulation of the Reticular Activating System.[1]

By

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Introduction.

This report gives an account of various electrographic effects in neuronally isolated cortex in the unanesthetized cat (cf. INGVAR 1955), following electrical stimulation of the reticular activating system of the brain stem (MORUZZI and MAGOUN 1949). It is concluded that the effects in the isolated cortex were caused by extraneuronal mechanisms, and some preliminary observations on their nature will also be reported.

Technique.

Unanesthetized cats with intact brain stem, immobilized with curare, were used. In another report, concerning the characteristics of the electrical activity of isolated cortex (INGVAR 1955), the details

[1] A preliminary report of this investigation was presented at the III International Congress of EEG in Boston, 1953 (INGVAR 1953) and at the C. I. O. M. S. Symposium on Brain Mechanisms and Consciousness in Ste. Marguerite, Canada, 1953 (INGVAR 1954).

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of the technique have been given. The evidence for the completeness of the neuronal isolation of the cortical slab was also presented in the same communication.

For the purpose of the present investigation, needle electrodes were placed stereotaxically in the reticular formation of the brain stem (usually at frontal plane + 2) and high frequency stimuli delivered over 5 to 15 seconds with a Grass rectangular wave generator. Usually a frequency of 200 to 250 c/s and a pulse duration of 0.5 msec. were used. The threshold voltage for obtaining the typical "arousal reaction" of the EEG varied in fresh preparations from 1.5 volts to values around 10 volts in later phases of the experiments. The stimulating electrodes were usually insulated from ground by a radio frequency isolation unit.

It became a major concern to show that the effects observed in the isolated area were not due to direct electrical spread from the point of stimulation, or due to stimulating effects from the recording electrodes. In several experiments, control stimulations of long duration and high intensity were carried out in various regions of the brain stem as well as in the hemispheres (even immediately underneath the isolated area) in order to ascertain the topographical specificity of the effects. It was not attempted to insert insulating material between the slab and the surrounding brain, since such a measure very likely would have jeopardized the circulation of the cortical slab. Indirect evidence, that the slab effects from reticular stimulation were caused by a specific mechanism, was obtained a) from the long latency of the effects, b) from the reproduction of some of the effects by carotid compression and, finally, c) from the fact that intercollicular brain stem section abolished the effects observed with a preserved arousal reaction in intact cortex (except in a few instances). The last finding (c) is furthermore inconsistent with the possibility that direct electrical stimulation of vessels could have accounted for the circulatory phenomena (see below), observed simultaneously with the cortical activation.

The cortical surface was studied through a microscope. Different areas were observed which were accessible to inspection with a wide craniotomy and a heated paraffin pool over the cortex. The magnification (approximately 30 ×) enabled the observer to follow the circulation of clusters of blood elements in small vessels. Venules or capillaries were studied generally. Since the vessels of the pia and those entering or leaving the cortex as well as the colour of the cortical background were observed, no distinction has been made here between the pial and the cortical circulation. The artificial respiration was regulated at the start of the experiment so that both hyperventilation (with vasoconstriction) and hypoventilation (with cyanosis and vasodilatation) were avoided (Schmidt 1936; Darrow and Graf 1945). No further analysis of the influence of respiration upon the stimulation results was carried out. It was attempted to influence the activity of the isolated cortex with periods of hyperventilation, but no clear results were obtained.

A gross control of the correlation between circulatory changes, ob-

served through the microscope, and the electrographic effects was obtained by taking down on the EEG record the findings of different observers at the microscope, these observers not being aware of when the stimulus was delivered (fig. 10).

It was found that a small change in the volume of the brain may accompany the reticular stimulation. Mechanical factors involved in the recording with silver or platinum wire electrodes — though very thin and flexible — were therefore eliminated in control experiments by the use of fine cotton wicks wrapped around the metal wires and soaked in saline. With such electrodes which gently rest on the cortical surface both the "suppression" as well as "desynchronization" slab effects, described underneath, were reproduced. The respiratory movements of the brain were very small with the wide type of craniotomy used (often combined with a drainage of the cerebrospinal fluid through the posterior fossa). No relation was found between the regular rate of the artificial respiration (approximately 20/minute) and the slab activity.

In most experiments the localization of the stimulated points was verified in histological sections of the brain stem.

Results.

It should be emphasized that the effects to be described were not always reproducible to the same degree in every experiment. Elsewhere (INGVAR 1955), the variabilities of the spontaneous and chemically induced slab activity and its causes have been discussed. Circulatory variations, to some degree depending upon the subpial dissection technique during preparation of the cortical slab, were mentioned. Such factors would determine the basic excitability of the slab which, in its turn, would influence the outcome of the reticular stimulation.

The effects of reticular stimulation upon the electrical activity of the isolated cortex.

The effects of reticular stimulation were studied upon spontaneous activity in the cortical slab of the "burst" type and upon chemically induced discharges of a more or less continuous type.

In 8 experiments electrical activity could be elicited in the isolated area by the reticular stimulation. In fig. 1 an example is given of this phenomenon. The latency of the appearance of the typical slab discharges was long and varied between 10 to 70

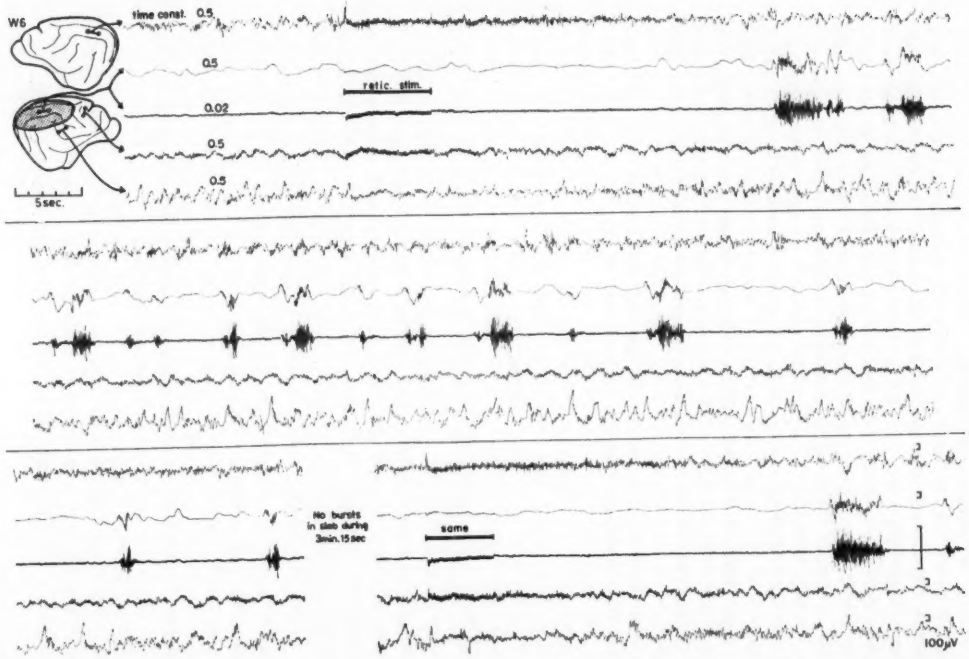


Fig. 1. Initiation of rhythmic activity ("bursts") in isolated cortex, previously electrically silent, following stimulation of the reticular activating system. Activity appearing in isolated cortex when the activation pattern in intact cortex is disappearing.

seconds from the start of the stimulation. It was possible to induce electrical activity of the slab both initially in the experiment (when the slab had not been active), as well as during later phases when the slab had become electrically inactive (as, for example, after long lasting periods of chemical treatment). In many instances, however, the reticular stimulation had no effect on the silent slab. In such cases 'subliminal' treatment with metrazol, applied locally, would occasionally render the reticular stimulation capable of starting an activity in the slab. The discharges then were of another type (fig. 2), bearing some resemblance to the one usually elicited by local metrazol.

Certain changes of the spontaneous bursts were frequently observed after reticular stimulation. In fig. 3 two examples are given of how the stimulation prolongs the bursts and lengthens the typical fast wave phase which usually initiates each burst. The stimulus in these cases was of approximately the same intensity and length, but it gave a more pronounced effect in the case where the activity of the intact cortex before the stimulation shows a less activated pattern (fig. 3 A). In other words, the slab effects seemed roughly proportional to the effects upon the EEG of the intact cortex.

When the slab activity was of the continuous type (spontaneously or chemically produced) there was often a more or less complete initial suppression of the activity, following the reticular stimulation. The latency of the suppression was about 1 to 5 seconds and a complete suppression could last for varying periods of up to 30 seconds. Once reestablished, the spontaneous bursts demonstrated the changes just mentioned. The silent periods between the bursts usually became somewhat shorter, which sometimes served to create the impression of a continuous activity.

Fig. 4 and 5 demonstrate various effects obtained from the brain stem stimulation upon metrazol discharges in the slab. It is seen that the initial suppression is followed by an "activation" or "desynchronization" of the pattern. There is a decrease of voltage and an increase of frequency of the high voltage potentials. The effects in the slab last for a certain period and the activity then slowly assumes a type very similar to the one before stimulation. Fig. 6 demonstrates another type of reaction of a delayed form.

In fig. 7 and 8 two examples are given of the effect of reticular stimulation upon "epileptic" discharges, *i. e.*, excessive

Fig. 1. Initiation of rhythmic activity ("bursts") in isolated cortex, previously electrically silent, following stimulation of the reticular activating system. Activity appearing in isolated cortex when the activation pattern in intact cortex is disappearing.

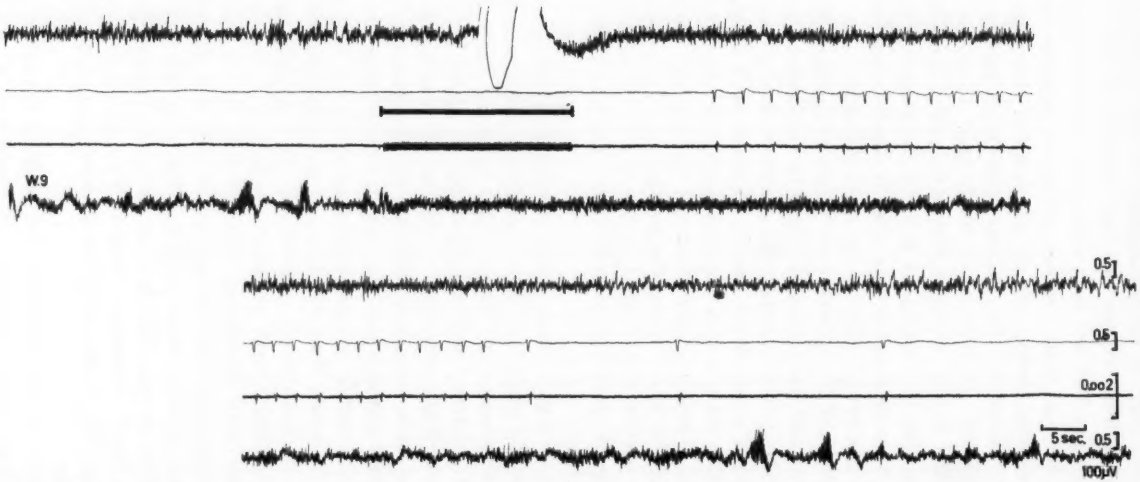
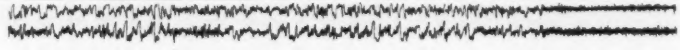


Fig. 2. Initiation of spike-like activity in isolated cortex (two middle channels) on reticular stimulation. The isolated slab treated with metrazol, applied locally, 1 minute before sample starts, after a period of depression with electrical silence. Recording as in figure 1, but with only one control lead (last channel) on intact cortex homolaterally. (Accidental mechanical artifact in upper channel during the reticular stimulation.)



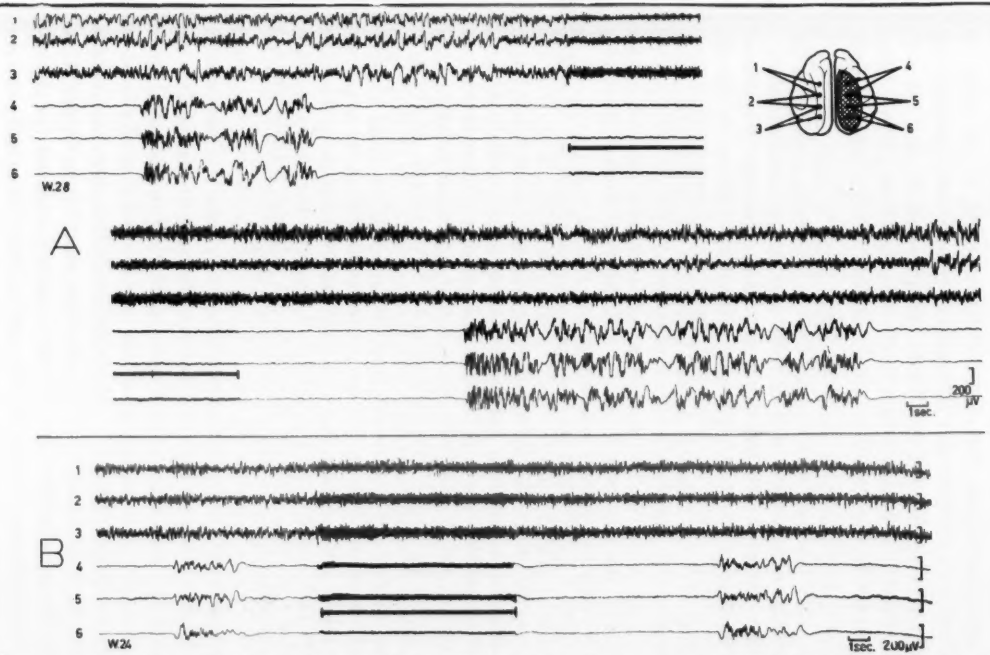


Fig. 3. Two examples of the effect of reticular stimulation upon typical bursts in isolated cortex. Note prolongation of the bursts and increase of fast activity in them after the stimulation. The stimuli in the two examples were of approximately the same length and intensity. More pronounced effects in the first example, 3 A, in which the intact cortex before the stimulation shows a less active pattern. Time constant here 0.5 seconds, in other figures marked on channels.

high voltage discharges, in the slab induced by metrazol locally. In fig. 8 the incipient seizure is suddenly brought to an end by the stimulation. The ensuing activity slowly decreases in amplitude and electrical silence follows after 120 seconds. In the other example (fig. 7) the reticular stimulation, in spite of repetition, has no effect upon the well developed epileptic discharges. The control leads in both cases demonstrate that the stimulation is effective in producing the activation pattern of the intact cortex.

Long lasting cortical depression states in the isolated area, as in fig. 8, were produced similarly by the reticular stimulation in a few other instances. In these cases the slab had usually been subjected to repeated chemical treatments. A depression, leading to electrical silence, could also be produced by mechanical trauma to the slab surface (fig. 9). In some instances it was observed that the depression travelled over the isolating section to involve the whole hemisphere. In intact cortex, however, a complete electrical silence, such as in the slab, never occurred. The depression with electrical silence could last in the slab for long periods of up to 20 minutes. During this time a refractoriness to chemical stimulation was present.

Without much doubt these phenomena represent cortical "spreading depression" (LEÃO 1944 a, 1947; LEÃO and MORISON 1945; SLOAN and JASPER 1950). Due to the fact that vascular (as well as volumetric) changes accompanied the electrographic effects, described above, which is also the case with "spreading depression" (LEÃO 1944 b; VAN HARREVELT and STAMM 1952), there is thus a possibility that the two phenomena may be functionally related. They cannot, however, be completely identical, since the circulatory as well as the electrographic effects, produced by the reticular stimulation, in the great majority of cases reversed fully in a few minutes at the most. It is possible that in the few instances in which depression was obtained, traumatic factors, involved in placing the chemical agent on the cortical surface, may have summed with the effects of the reticular stimulation with a consequent depression (see MARSHALL 1950).

It will be apparent from the illustrations that the earliest slab effects had a longer latency than the effects in intact cortex. An exact determination was not attempted due to the method of recording and the variations of the slab activity at the start of the stimulation. Effects in the slab were seldom, if ever, recorded within the first second or two after the start of the stimulation.

The duration of the slab effects usually paralleled the activation in intact cortex very closely and it is seen, especially in fig. 4 and 5, that the "desynchronization" comprised the whole of the cortex including the isolated part.

Observations on the cortical surface.

Microscopical observation of the cortical surface revealed that changes in the pial circulation were produced by the reticular stimulation (fig. 10). These changes were observed both over intact as well as over isolated cortex, and the description, to be given below, therefore applies to both. It has been realized, that the subjective method of observation used is very gross. It has not permitted the establishment of close time relations or quantitative or regional differences (see SCHMIDT 1936).

In the resting period, *i. e.*, when no stimulation had been carried out for several minutes, the cortical surface was usually characterized by a rather pale background against which arteries and veins could be differentiated easily by their colour. The speed of the circulating blood elements in the capillaries was not more than a few millimeters per second.

Initially, when the reticular stimulus was applied, either the whole picture remained unchanged for a period of 1 to 5 seconds or (upon higher intensities of stimulation?) — in some cases only — a certain blanching of the background was seen. This usually took place after 1 or 2 seconds following the start of the stimulation and lasted a similar period. A sudden arrest of the circulating blood elements was often seen simultaneously in the small vessels.

After this period the circulation started again, often at first with the blood corpuscles passing in another direction than previously, but then, after an initial 'hesitation', they began to stream at rapidly increasing speed in the original direction. The speed often changed so as to make it impossible to distinguish individual blood elements, even within quite small vessels. During the phase of increasing circulation the background colour of the cortical surface often changed appreciably, giving the impression of a reddish 'veil' being pulled over the background. This happened about 3 to 5 seconds after the stimulus was applied. Furthermore, it was often clearly seen that the larger and the smaller arteries and veins dilated. Very often, also, new cortical capillaries, not seen previously, opened up. When no blanching occurred the picture changed directly into the one of circulatory increase.

Concomitant with the increase in circulation the slight volume change, mentioned above, took place, and the electrographic slab effects were seen. This phase in circulation lasted undiminished for a short period, gradually to subside during the following 30 to

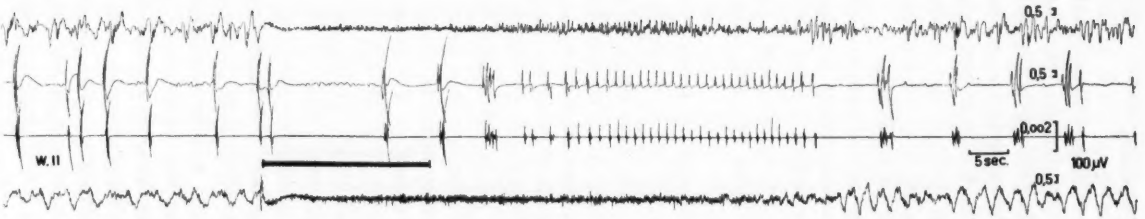


Fig. 4. "Desynchronizing" effect on the electrical activity of isolated cortex (induced with metrazol locally) following reticular stimulation. Note that the slab effects last as long as the "desynchronization" lasts in intact cortex. Cervical sympathetic nerves sectioned. Recording as in figure 2.

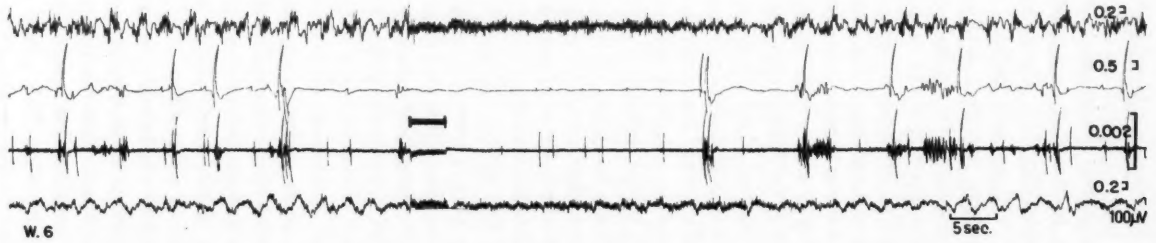


Fig. 5. Suppressing effect on the electrical activity of isolated cortex (induced with metrazol locally) on reticular stimulation. Recording as in figure 2.

Fig. 5. Suppressing effect on the electrical activity [illegible] Recording as in figure 2.

Fig. 6. Delayed type of effect upon metrazol induced activity in isolated cortex on reticular stimulation. During 14 minutes preceding sample a large dose (35 mg/kg) of hexamethonium bromide was injected intravenously. Recording as in figure 1, but with the electrocardiogram on last channel.

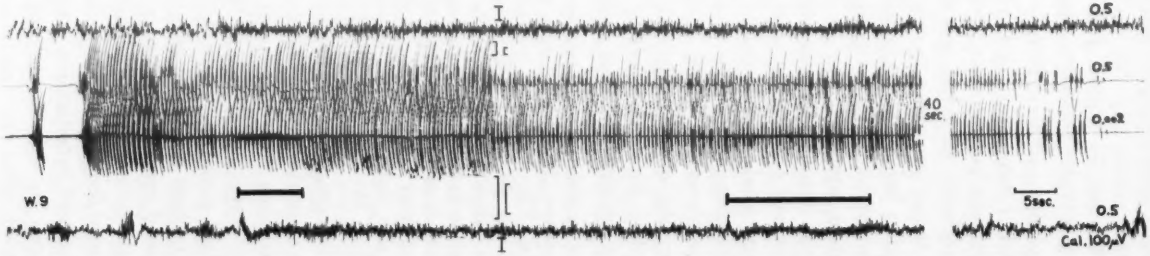


Fig. 7. No effect of reticular stimulation (repeated twice) upon excessive "epileptic" discharges induced with metrazol in isolated cortex. The first stimulation is capable of activating intact cortex. Hexamethonium bromide injected intravenously during 8 minutes before sample starts. Another sample taken later in the same experiment (figure 6) demonstrates that the injection was not capable of blocking the slab effects from reticular stimulation. Recording as in figure 2. Amplification diminished according to calibrations.

90 seconds. This regression and the EEG changes often paralleled each other closely (fig. 10).

The circulatory effects were studied repeatedly in 19 experiments and the principal events were all reproduced in a more or less pronounced way. Different preparations showed a different threshold of stimulation, and there did not seem to be any threshold difference between the circulatory changes and the electrographic effects. As in the case of reticular activation of the EEG, it was found necessary to wait several minutes between each stimulation before it was possible to reproduce to its full extent the cortical circulatory changes previously seen at the same stimulation intensity.

In some instances spontaneous changes in the vessel diameters (DARROW and GRAF 1945) were observed, but these were always small and local. Phenomena of the type described such as the 'veil' phenomenon were never seen to occur spontaneously. In a few experiments in which adequate stimuli were tested, some electrographic effects in the slab could be obtained by nociceptive stimulation. No certain cortical circulatory changes could, however, be established microscopically.

In the experiments differences in the "basic" circulatory picture were observed. In some a rapid, in others, a slow cortical circulation was seen. The impression was gained that in intact cortex rapid circulation was associated with a more activated pattern in the EEG, slow circulation with a slow wave picture with spindles. This impression is in accordance with observations by DARROW and GRAF (1945) who used an objective method of recording the cortical blood flow. The circulatory changes and, as mentioned, the electrographic effects in the slab upon reticular stimulation were often found to be pronounced in the first case and less so in the latter.

The topographical localizations of the stimulation points, yielding the circulatory changes, have been summarized in fig. 11. It is seen that they lie in those parts of the brain stem ascribed to the reticular activating system in the cat (STARZL, TAYLOR and MAGOUN 1951 a and b). There were, however, some points (marked with X) which gave only an activation pattern and a much less pronounced cortical circulatory reaction or none whatever, when identical recovery periods were used between the stimulations. Such points were found in two experiments in which a more systematic topographical investigation was carried out. No clearly defined differences in the electrographic activation patterns could be established, when the circulatory changes were present or absent in these instances.

Preliminary observations on the nature of the cortical circulatory changes following reticular stimulation.

The stimulation points, yielding the cortical circulatory changes, were situated in regions which are known to give rise to pressor effects (KABAT, MAGOUN and RANSON 1935; HODES and MAGOUN 1942; HESS 1949; DELL 1952; see Addendum). It is furthermore known that changes in the blood pressure may cause changes in the cerebral circulation (see SCHMIDT 1936, 1950). It was thus possible that one part of the effects, observed in the isolated cortex, were due to changes in the systemic circulation following the reticular stimulation. The 'veil' phenomenon suggested, however, that cortical vasomotor mechanisms could have been actively participating. Some gross factors, influencing the blood pressure, were therefore eliminated in order to obtain preliminary information on this point.

Section of the spinal cord at C 1 (2 experiments), section of the cervical sympathetic nerves (5 experiments), the vagi (3 experiments) as well as injections of hexamethonium bromide (in 2 experiments in doses of up to 40 mg/kg during 5 to 8 minutes, in order to block sympathetic ganglionic transmission; PATON and ZAIMIS 1951; POOLE 1954), were all measures which seemingly, either individually or in various combinations, left the cortical circulatory changes unaffected as far as could be distinguished under the microscope. Adrenalectomy was performed in one experiment, but it did not change the electrographic or circulatory effects. In several experiments, *e. g.*, no. W9 (fig. 6 and 12) the electrocardiogram was recorded, but no changes were observed in the heart rate during or after reticular stimulation.

In three preparations an intercollicular brain stem section (fig. 11) was performed. In these experiments the circulatory changes, previously seen after reticular stimulation, disappeared and remained absent during the following period of observation which lasted several hours. The EEG of intact cortex thus attained the type usually met with in *cerveau isolé* preparations (BREMER 1935, 1938). It could, however, still be activated by reticular stimulation, carried out rostrally to the section. Attempts to elicit cortical circulatory changes by brain stem stimulations caudally to the section were made in a few instances with negative result.

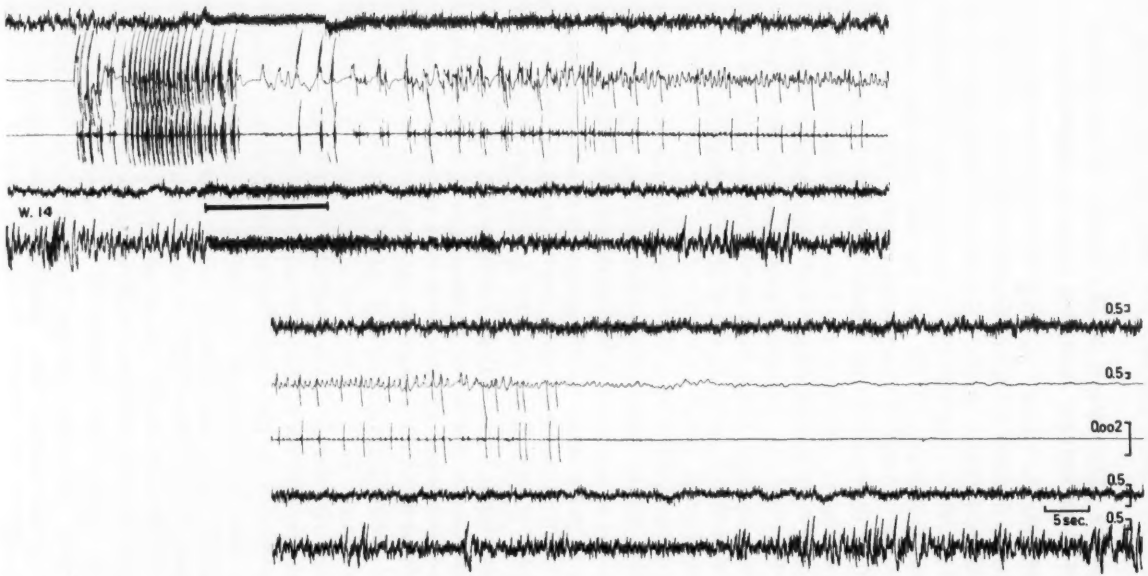


Fig. 8. Effect of reticular stimulation upon metrazol induced "epileptic" discharges in isolated cortex. There is a "desynchronization" of the pattern in the isolated slab followed by a diminution of voltage with an ensuing electrical silence due to cortical depression. The depression in this case remained localized to the isolated area. Recording as in figure 1. Cal. 100 μV.

of the pattern in the isolated slab followed by a diminution of voltage with an ensuing electrical silence due to cortical depression. The depression in this case remained localized to the isolated area. Recording as in figure 1. Cal. 100 μV.

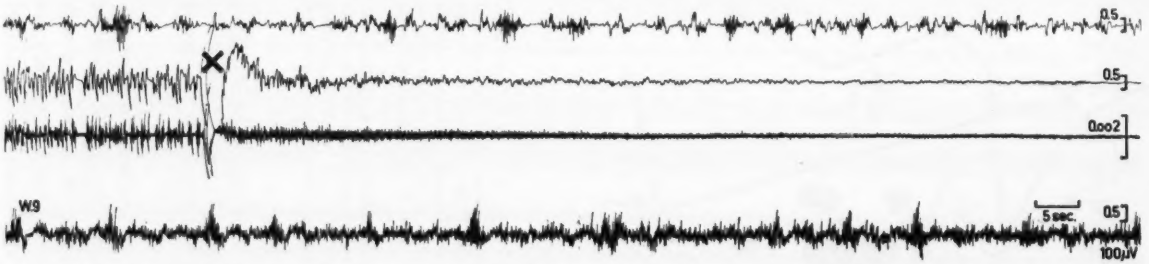


Fig. 9. The effects upon the electrical activity of isolated cortex from a slight mechanical trauma (×) delivered to the electrodes recording from it. There is a depression followed by electrical silence. Recording as in figure 2.

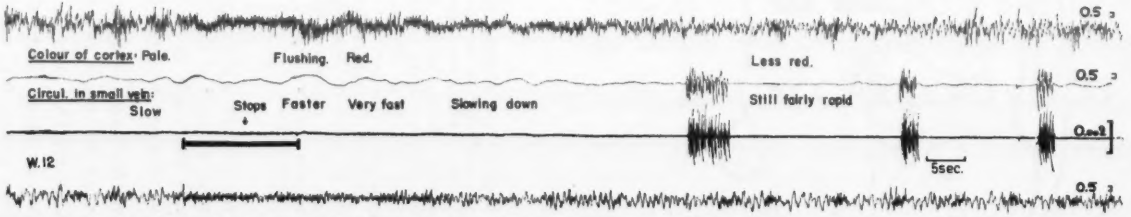


Fig. 10. Gross correlation between subjective microscopical observations on the cortical surface and electrographic events during and after reticular stimulation. Recording as in figure 2. Cal. 100 μV.

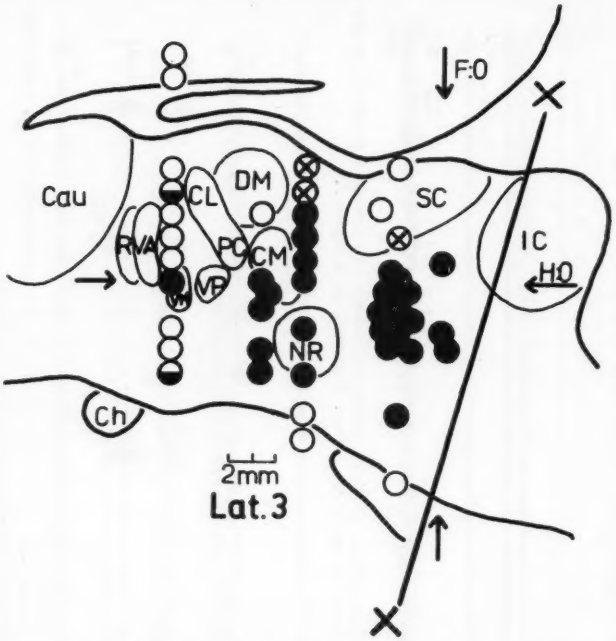


Fig. 11. Diagram of a parasagittal section of the brain stem of the cat, summarizing the topographical localization of stimulation points which gave electrographic as well as circulatory effects (filled circles). Half filled circles indicate less pronounced effects. Crossed circles indicate electrographic activation effects without any microscopically visible circulatory changes on the cortical surface. Empty circles indicate no effects. × ——— × line of brain stem section in intercollicular plane (see text).

Cau n. caudatus; Ch chiasma; CL n. centralis lateralis; CM n. centrum medianum; DM n. medialis dorsalis; IC inferior colliculus; NR n. ruber; PC n. paracentralis; R n. reticularis; SC superior colliculus; VA n. ventralis anterior; VM n. ventralis medialis; VP n. ventralis posterior. Frontal and horizontal planes 0 as indicated.

In a few instances in these experiments some vague and ill-defined *electrographic* changes of the suppression type with long latency (10 to 20 seconds) were seen in *the isolated area* in spite of the absence of circulatory changes on microscopical observation.

The electrographic suppression effect in the isolated area suggested that an acute decrease of cortical circulation could be responsible for this part of the phenomena. In accordance with this interpretation, bilateral compression of the carotid arteries for a short period was performed in 3 experiments. This caused

Fig. 12. Two samples from the same experiment demonstrating the similarity between the effects of A) reticular stimulation and B) compression of the carotid arteries bilaterally, upon the electrical activity of isolated cortex. In B there is no activation of the intact cortex, but instead a depression. No changes in heart rate in either example. Recording as in figure 1, but with the electrocardiogram on last channel.

an arrest of the circulation in the smaller cortical vessels, and it also reproduced the suppression effect in the slab. Fig. 12 A and B demonstrate the similarity between the slab effects from reticular stimulation and those from carotid compression in the same experiment. In the latter case (fig. 12 B) there is in intact cortex a change of the cortical activity with spindles instead of an activation as in the former case (fig. 12 A). It is believed that the carotid compression was able to reproduce at least one part of the circulatory phenomena accompanying the cortical activation.

As described (BURNS 1950, 1951; INGVAR 1955), barbiturates, even in small doses (5 to 10 mg nembutal or 10 to 15 mg pentothal) intravenously, were found to cause electrical silence in the isolated cortical area. During the experiments it was also confirmed that these anesthetics, by blocking the reticular activating system (FRENCH, VERZEANO and MAGOUN 1953 b), make it impossible to elicit the cortical arousal pattern on electrical stimulation of the brain stem. It was furthermore found that the barbiturate anesthesia abolished the cortical circulatory changes which follow such stimulation in the unanesthetized preparation.

Deteriorated preparations resembled the anesthetized ones in that the electrographic and circulatory changes on stimulation of the brain stem could not be elicited. These findings confirm the impression that the reticular activating system is a sensitive functional entity with a limited capacity in the experimental situation chosen (HUNTER and INGVAR 1955).

On some occasions observations were made in the microscope simultaneously with the injection of the anesthetic agent. During the acute change of the EEG into the typical barbiturate pattern there was often a decrease of the speed of circulation and in the anesthetic state the cortical circulation remained slow (*cf.* SEROTA and GERARD 1938).

From a number of other scattered observations it may be mentioned that CO_2 was found to block all activity in the isolated area. Many different concentrations were administered in air or oxygen with the same result. It was therefore not possible to investigate the effects of reticular stimulation upon an electrically active isolated area in which a CO_2-vasodilatation had been induced. Ephedrine, injected in a small amount intravenously, gave rise to an activation pattern in the EEG, confirming LONGO (1954), and also gave an increase in speed of circulation. For a period after the injection no circulatory effects could be elicited by the reticular stimulation and the electrographic effects were small.

Discussion.

Some general implications of the findings for features of the electrical activity of normal and isolated cortex have been discussed elsewhere (INGVAR 1955). The electrographic data seem to have several implications, and therefore some pertinent studies of circulation and electrical activity of the brain will be briefly reviewed and related to present concepts of the reticular activating system.

Changes in the cerebral circulation are in general considered to take place through either local-humoral or general-neural mechanisms (see SCHMIDT, 1950).[1] Several authors (COBB and TALBOT 1927; FULTON 1928; SCHMIDT and HENDRIX 1937; SEROTA and GERARD 1938) have chiefly investigated mechanisms of the first category. These authors demonstrated with various methods that sensory stimulation is accompanied by an increase in the cerebral circulation which is localized to structures specifically involved in the modality tested. There is reason to believe (SCHMIDT 1950) that metabolic products from increased neuronal activity account for such local effects.

With the thermoelectric method devised by GIBBS (1933), GIBBS, LENNOX and GIBBS (1934) and PENFIELD, VON SANTHA and CIPRIANI (1939) demonstrated an increase in the cerebral circulation during epileptic discharges. VON SANTHA and CIPRIANI (1937) extended these studies to demonstrate topographically localized subcortical changes in the circulation after local electrical stimulation of the cortex. JASPER and ERICKSON (1941) were the first to carry out a simultaneous study of the EEG, blood flow and pH in a cortical area in which excessive discharges were produced by means of metrazol or electrical stimulation. These authors concluded, as did GIBBS et al. and PENFIELD et al. that the increase in circulation (accompanied by vasodilatation) was secondary to the epileptic manifestations, but that circulatory changes constituted an important factor for the maintenance of cortical homeostasis.

Mechanisms of the other category, the general-neural, regulating the cerebral blood flow, have only been correlated with the EEG by a few investigators.

[1] The important rôle played by the CO_2 concentration of the blood for the cerebral vasomotor tone is beyond the scope of the discussion.

In a series of papers, DARROW and his collaborators have analyzed the relation between the vasomotor control of the cerebral circulation and the EEG. DARROW and GRAF (1945) recorded the cortical circulation in cats (unanesthetized or under light dial anesthesia) with a photoelectric method. They found dilatation to be associated with "an increase of frequency, increased potential of fast waves and decreased slow activity". Paling or vasoconstriction, on the other hand, was associated with "decrease of frequency, increase of potential of slow waves and decreased fast activity". DARROW, GREEN, DAVIS and GAROL (1944) demonstrated the regulatory influence upon brain potentials from the parasympathetic innervation of the cerebral vessels along the greater superficial petrosal nerve, a pathway originally described by CHOROBSKI and PENFIELD (1932).

The studies quoted have all emphasized the intimate relationship between neuronal functions (normal as well as pathological (epileptic)) and circulation in the central nervous system. The present findings have demonstrated (with a subjective method) that the change of cortical electrical activity, following reticular stimulation, is also accompanied by cortical circulatory changes.

It should be emphasized that the results thereby confirm and elaborate previous studies (*without EEG observations*) of the cortical circulation, following electrical stimulation of the brain stem. FOG (1934) found pial vasodilatation on electrical stimulation of mesencephalic and diencephalic structures in cats which were or were not accompanied by blood pressure changes. He concluded that the pial vascular changes were elicited through cranial nerves (*e. g.*, in the greater petrosal superficial nerve, discussed above). STAVRAKY (1936) also reported various forms of vascular changes in the pia on electrical stimulation of the hypothalamus and the thalamus which in several ways coincide with the observations reported above. He concluded that these structures exert an influence upon the pial vasomotor tone.

Several studies in recent years, particularly by MAGOUN, JASPER and DELL and their collaborators (JASPER 1949; MORUZZI and MAGOUN 1949; STARZL, TAYLOR and MAGOUN 1951 a and b; DELL 1952; FRENCH, VERZEANO and MAGOUN 1953 a; HANBERY, AJMONE-MARSAN and DILWORTH 1954) have analyzed the neuronal mechanisms by which the mesencephalic and diencephalic parts of the reticular system exert their wide-spread influence upon the electrical activity of the cortex. In view of the effects, obtained

in isolated cortex from stimulation of the brain stem, it would seem necessary to conclude that the reticular activating system — in the unanesthetized state — also influences the "cortical excitatory state" (JASPER 1936) or *tonus cortical* (BREMER 1938, 1949) by means of extraneuronal mechanisms.

The results suggest an intimate relationship between the circulatory changes, observed microscopically on the cortical surface, and the electrographic effects in the isolated cortex. At present, however, the nature of the circulatory changes appears complex. One factor may pertain to changes in the systemic circulation caused by the stimulation of the brain stem (see Addendum). (Circulatory changes, due to alterations of the tone of respiratory muscles simultaneously with the stimulation, cannot have been of importance since curarized preparations were used; SCHMIDT 1936.) Cortical vasomotor processes may also, it seems, actively participate, since cortical circulatory changes persisted after various procedures eliminating pressor effects, but were abolished by brain stem section at the intercollicular plane. The latter procedure would also exclude pituitary hormonal effects.

It must be stated, however, that there is no unequivocal evidence to prove that the circulatory changes observed were the real cause of the electrographic effects recorded in the isolated cortex. The findings do by no means exclude that circulatory as well as the electrographic slab effects are based upon humoral events of a local-metabolic or a more general character. This alternative explanation is, however, hypothetical. The exact nature of the extraneuronal effects upon isolated cortex therefore remains unknown at present.

From a clinical EEG point of view, the findings in isolated cortex (fig. 3 to 8) indicate that extraneuronal mechanisms, mediated through the reticular activating system, may exert an influence upon epileptic discharges in the cortex of a precipitating as well as a suppressing type. Such effects will, however, ultimately depend upon the excitability conditions present in the cortex (changed with metrazol in the experiments) at the moment of reticular activation. Apparently the influences upon epileptic phenomena have a limited range and will not influence well developed epileptic discharges, a statement supported by the experimental findings of MORUZZI and MAGOUN (1949) and of LAIRY-BOUNES, PARMA and ZANCHETTI (1952).

The results may therefore contribute to an explanation of how cerebral circulatory factors influence epileptic manifestations (PENFIELD 1938; PENFIELD and JASPER 1954). They may, it seems, especially provide additional information for the understanding of the effects of arousal upon various epileptic abnormalities of the EEG (GELLHORN and BALLIN 1948; ARDUINI and LAIRY-BOUNES 1952; GIBBS, and GIBBS 1952; LAIRY-BOUNES, PARMA and ZANCHETTI 1952; LI, JASPER and HENDERSON 1952).

ADDENDUM.

Since the main part of the present investigation was accomplished, the report of BONVALLET, DELL and HIEBEL (1954) has appeared. They showed that after prebulbar section sciatic stimulation (*i. e.*, nociceptive stimulation) was still capable of producing cortical activation. Activation could also be produced by stimulating the peripheral cut ends of the splanchnic nerves or by injection of adrenaline. By various means it was demonstrated that cortical activation upon nociceptive stimulation takes place a) through an immediate neuronal mechanism operating through the ascending reticular activating system and b) through a humoral (adrenaline) activation, not acting directly on the cortex, but through pontomesencephalic structures. Distension of the carotid sinus produced a suppression of the cortical activity, an effect mediated through purely nervous mechanisms. An increase in the blood pressure was not found to exert an activating influence directly upon the cortex. It was concluded that "the level of peripheral sympathetic tone is just as important a factor in maintaining the waking state as the continuous inflow to the brain of proprioceptive and exteroceptive stimuli".

The findings of BONVALLET et al. also clearly demonstrated that an increase in the blood pressure may accompany electrical stimulation of the (ventral portions of the) mesencephalic reticular system. The evidence has been reviewed above which supports the assumption that blood pressure changes cannot altogether have been responsible for the effects reported in the present investigation.

It would seem that the results of BONVALLET et al. and those reported above elucidate aspects of the interaction between autonomic and humoral mechanisms and the reticular activating system of the brain stem.

Summary.

1. Electrographic evidence is presented to show that the spontaneous as well as the (chemically induced) epileptic electrical activity of neuronally completely isolated parietal cortex of the cat may be influenced by electrical stimulation of the reticular

activating system of the brain stem. A description is given of various forms of effects recorded.

2. By gross microscopic observation it was found, on intact as well as on isolated cortex, that cortical circulatory phenomena accompany the EEG activation elicited by reticular stimulation. A description is given of these phenomena, the most conspicuous feature of which was a vasodilatation and an increase in the speed of circulating blood elements.

3. Subjectively, there seemed in general to be a direct proportionality between the electrographic effects in isolated cortex and the circulatory changes. The circulatory changes often closely paralleled in time the electrographic effects in isolated cortex (as well as the electrographic changes in intact cortex).

4. The grossly visible cortical circulatory changes on reticular stimulation were abolished, together with activation of the EEG, by small doses of barbiturates given intravenously.

5. The mechanisms underlying the circulatory changes have been analyzed in a preliminary way. Such changes, as well as electrographic effects in isolated cortex, were observed after various procedures interfering with possible pressor effects caused by the reticular stimulation. The grossly visible cortical circulatory changes on tegmental and diencephalic reticular stimulation were abolished by intercollicular brain stem section, a procedure, however, which did not interfere with the electrographic activation of intact cortex.

6. The effects of reticular stimulation upon chemically induced epileptic discharges in isolated cortex may provide additional information for the understanding of the effects of arousing stimuli upon epileptic abnormalities in the EEG.

7. The results suggest that the reticular activating system of the brain stem may influence cortical electrical activity by means of extraneuronal mechanisms. The findings indicate that circulatory factors are involved, but it is also possible that humoral events may be primarily responsible for the effects recorded.

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Influence of Visual Cortex on Light Impulses in the Brain Stem of the Unanesthetized Cat.

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Introduction.

Studies with electrophysiological methods have demonstrated important afferent pathways to the cerebral cortex which are distinct from the classical 'lemniscal' routes (DERBYSHIRE, REMPEL, FORBES and LAMBERT 1936; DEMPSEY, MORISON and MORISON 1941). Anatomical and physiological evidence support the view that sensory impulses arrive at the reticular activating system of the brain stem (MORUZZI and MAGOUN 1949) by means of collaterals from the *specific* pathways and that they, from there on, are transmitted to the cortex through *unspecific*, probably polysynaptic connections. In numerous publications MAGOUN, JASPER and DELL and their collaborators have established the functional significance of the mesencephalic and the diencephalic portions of the reticular system and the importance of the unspecific cortical afferent connections (JASPER 1949; MORUZZI and MAGOUN 1949; STARZL and MAGOUN 1951; STARZL, TAYLOR and MAGOUN 1951 a and b; STARZL and WHITLOCK 1952; FRENCH, VERZEANO and MAGOUN 1953 a and b; HANBERY and JASPER 1953; MAGOUN 1953; HANBERY, AJMONE-MARSAN and DILWORTH

This investigation was presented at the XIX International Physiological Congress in Montreal 1953. (INGVAR and HUNTER 1953.)

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1954). Recently, DELL (1952) has given an extensive review of the field which also summarizes his own contributions and those of his collaborators.

Investigations by a number of workers (GERARD, MARSHALL and SAUL 1936; GASTAUT, NAQUET, CORRIOL, ROGER and BADIER 1950; HUGELIN, BARTHIER, BONVALLET and DELL 1952; BONVALLET, DELL and HUGELIN 1952; FILLENZ 1953; FRENCH, VON AMERONGEN and MAGOUN 1952) have demonstrated with physiological methods the existence of unspecific pathways in the brain stem for the visual modality. The importance of such pathways for induction of epileptic phenomena by means of photic stimulation has been studied experimentally by GASTAUT and HUNTER (1950) and more recently by HUNTER and INGVAR (1955)[1].

Psychophysiological as well as experimental evidence (RHEINBERGER and JASPER 1937; JASPER and SHAGASS 1941; SLOAN and JASPER 1950; KAADA 1951; PENFIELD and JASPER 1954) has, however, indicated that cortical mechanisms would also be engaged primarily in controlling the effects from unspecific systems of the brain stem. In an experimental study in the monkey, JASPER, AJMONE-MARSAN and STOLL (1952) demonstrated the existence of corticofugal pathways to regions in the brain stem which are also ascribed to the reticular activating system. BREMER and TERZUOLO (1953) have shown that impulses originating in the cortex may have an alerting effect upon a sleeping animal. The investigations of LIVINGSTONE, HERNANDEZ-PEON and FRENCH (1953) and of BUSER, LIVINGSTONE and FRENCH (1953) have further emphasized the corticofugal pathways to the reticular system.

The present report concerns the importance of the visual cortical projection areas in the cat (see Technique) for the unspecific visual pathways of the brain stem. Elsewhere (HUNTER and INGVAR 1955), it has been demonstrated that "irradiated" visual impulses, induced under metrazol, are transmitted to the frontal cortex more rapidly, if the visual cortical areas are present than if they have been ablated. These observations were made in lightly anesthetized preparations. The present study has been made in unanesthetized cats. It deals with the topography and the latencies of subcortical responses to low frequency light stimulation. In one group, preparations with intact

[1] Reference is made to this report for a review of other investigations on the "photo-metrazol activation technique" in clinical EEG.

brains have been used, in a second group the visual cortex had been previously ablated. In many respects the results confirm previous studies (referred to above) on the visual modality. In addition the findings suggest the possibility that the visual cortex on visual stimulation may control events elicited simultaneously in the brain stem.

Technique.

Eleven mature cats were used which were prepared under ether. The exploration was then carried out (not less than 1 hour later) in preparations immobilized with curare and maintained with artificial respiration. Local anesthesia was applied around the edges of the exposure which usually displayed both hemispheres. Bilateral cochlear destruction was made, and the pupils were dilated with homatropin. High intensity, low frequency light stimulation was provided by flashes from a stroboscope discharge tube, mounted 30 centimeters in front of the animal's head which was placed in a stereotaxic instrument (*cf.* HUNTER and INGVAR 1955).

Four cats with intact brains were explored ("intact series"). In 3 cats ("ablated series") a bilateral ablation of the visual cortical projection areas (POLYAK 1927; TALBOT 1940; MARSHALL, TALBOT and ADES 1943; see also DELL 1952) and some adjacent cortex had been made (44, 14 and 11 days) previously under nembutal. The ablation had been carried rostrally to about 5 millimeters caudally to the posterior sigmoid gyrus and it involved the entire occipital pole (the lateral and postlateral gyrus, the middle and the posterior suprasylvian gyri). All grey and white matter had been removed down to the depth of the sulci (sulcus intercalatus, sulcus suprasylvius posterior and medius) surrounding the gyri concerned. Before subcortical exploration was made in these preparations it was determined that no evoked potentials from visual stimulation could be obtained over the area of removal.

In one cat, originally planned for control purposes, the auditory areas had been ablated bilaterally. On subsequent exploration it was found, however, that small visual evoked responses could only be obtained from a limited area in one of the hemispheres. This indicated that the geniculo-cortical bundle (POLYAK 1927) had been severed (completely on one side and almost completely on the other) by the temporal ablation which had been carried too deep. This was later confirmed by the histological control. Since the results from this cat (on exploration in hemisphere without cortical visual responses) coincided with those from cats with complete ablation of visual areas, this experiment was included in the ablated series.

A uni-lateral ablation of the visual areas was made in two preparations and also an experiment with (acute) bilateral removal of the temporal lobes (see Results).

Subcortical exploration was made with a 4-needle electrode (needle

diameter 0.4 mm). All needles were pointed and insulated to their tips (50 to 100 μ) by means of a plastic varnish. They were arranged in a single line in a frontal plane. The same electrode was used in all experiments. The interelectrode distance was 1.3 mm. The structures to be explored were approached by stereotaxic technique from above in different frontal planes and with different lateral electrode settings. Thus, the regions covered were from frontal plane 0 (the interauricular plane) to + 15 with preference for structures adjacent to the midline between frontal planes + 2 to + 12.

The potentials recorded (between two adjacent electrode tips, *i. e.*, bipolar recording in three pairs) were fed to Offner condensor coupled preamplifiers (time constant 0.2 to 0.02 seconds) connected to a 5-beam oscilloscope (good frequency response up to 10 KC). A control lead from the surface of the visual cortex was used in the intact series. The sweep was triggered by the shutter of a camera, the light flash of the stroboscope being discharged at the same time by a delay circuit. The 4-needle electrode was lowered millimeter by millimeter, and oscillographic records were taken at each level. The light flashes were repeated by operating the camera shutter manually at a frequency of about one flash per two seconds. The responses were observed on the oscilloscope screen (no light in room) and between 5 and 10 records were taken from each electrode position. In attempts to limit the number of photographs, fewer records were taken from electrode placements where large and constant responses were present, *e. g.*, in the optic tract.

The electrode tracks were identified in serial histological sections of the brains (frozen or paraffin sections in Nissl and Weil stains) and plotted into brain charts (10 times enlargement) of corresponding frontal planes based upon the stereotaxic atlas of JASPER and AJMONE-MARSAN (1954). Usually, in each electrode position two small electrolytic deposits of iron had been made (stained by Prussian blue) with varying electrode tips at different levels. From a comparison of these marks with notes on the level of the brain surface and that of bony resistance it was possible to extrapolate the electrode positions in the individual brain charts. It is believed that by this method the error of localization has not exceeded 1 mm.

The latencies of the responses recorded were measured from the artifact of the flash to the first constant deflection in the photographs obtained from the oscilloscope (time scale in 5 msec. intervals). The average value of the latency of the responses at each electrode position was then noted in the corresponding position of the brain charts. Control measurements gave variation of $\pm$ 5 msec. for the smallest responses and $\pm$ 2 msec. for medium sized and large responses. The summarizing diagrams of fig. 3 and the parasagittal plottings of fig. 5 to 8 are based upon brain charts of individual experiments. Gross average latencies in certain subcortical fields (large numbers in fig. 3 A, B and C) were calculated from the individual latencies in corresponding fields in each experiment.

Results.

Potential pattern. A comparative study of visual subcortical responses recorded with bipolar technique (recording between two adjacent needle tips) and so called monopolar technique (reference electrode on the occipital bone) demonstrated that the monopolar method was not suited for the present investigation. With widely spaced leads, a potential (latency 10 to 15 msec.) was recorded in most places in the brain (fig. 1). It is assumed that this potential which often was W-shaped is the same as the one described by MARSHALL (1949) and CHANG and KAADA (1950) who studied cortical responses to electrical stimulation of the optic nerve. CHANG and KAADA state that this potential "was caused by the potential difference of an electrical field set up by the synchronous volley of impulses passing along the optic nerve and tract in a volume conductor". Since it probably lacks physiological significance, and since it highly minimized the differentiation of other responses, the bipolar technique was chosen for the present study. It is realized that the bipolar technique has disadvantages of its own. The needle tips may be isoelectrical when situated in large structures which act in synchrony and "false" latencies may be obtained. The great number of observations made in different animals have, however, demonstrated certain consistent tendencies which coincide between the animals in the same group. In the following discussion only such tendencies will be dealt with.

In fig. 1 and 2, topographical plots have been made of responses obtained with monopolar and bipolar recordings respectively, in a frontal plane (F + 7.5) in the same experiment from the ablated series, with the same electrode placement. The principal structures of the section are indicated in fig. 2 at the height of their centers. In bipolar recording (fig. 2) a distinct field of well defined potentials is apparent in an area surrounded from above by n. lateralis dorsalis (LD) and n. medialis dorsalis (DM), by n. centrum medianum (CM) medially and by n. ventralis posterior (VP) from below. In monopolar recording (fig. 1), due to the facts just mentioned, the same responses are less well differentiated.

In both fig. 1 and 2, a high-voltage rhythmic potential is seen from the Ammon's horn (5th response from below in second row of responses from the left, response just below large volleys from optic tract, OT). Such potentials were noted by RENSHAW, FORBES and

MORISON (1940) in microelectrode studies of this structure and ascribed to injury. In the present study these discharges served to control the electrode position during the experiment. When they were seen the exploring electrode was kept stationary until they had disappeared and a regular baseline permitted verification of responses. Definite responses to light were, however, never obtained (by the bipolar technique) from the hippocampus proper or from the fimbria hippocampi or fornix. This negative finding is at variance with observations by GERARD, MARSHALL and SAUL (1936) and ROBINSON and LENNOX (1951). More knowledge of differences in techniques employed would be necessary to explain the discrepancy.

Differences between intact and ablated series. In both series responses to visual stimulation could be recorded from wide areas outside the classical visual pathways in mesencephalic, diencephalic and also telencephalic structures. The results thereby principally confirm the observations of STARZL, TAYLOR and MAGOUN (1951 a and b) and those of FRENCH, VERZEANO and MAGOUN (1953 a) by the fact that unspecific brain stem responses are not unconditionally dependent upon the presence of the cortical projection area of the modality tested.

The responses in the intact series, especially in the rostral parts of the diencephalon, were usually of lower voltage and somewhat less wide-spread than in the ablated series (see Discussion). Differences between the two series in the distribution of responses and magnitude of latencies will be apparent from fig. 3 A, B and C as well as from the parasagittal plots in fig. 5 to 8.

In the *ablated series* the parasagittal plots (fig. 6 and 8) demonstrate the inflow of visual impulses from the optic nerves and tracts into the region of the lateral geniculate body, the pretectal area and the colliculus superior. There is an area of larger responses (= large numbers) which were recorded throughout the thalamus and the subthalamic region with increasing latencies in rostral direction. This "pathway" is best seen in the parasagittal plot of fig. 6. It goes ventrally to a region occupied chiefly by the n. medialis dorsalis and n. lateralis dorsalis where some small responses with a relatively short latency are seen. In a more lateral region (fig. 8), these early responses were larger close to the lateral geniculate body.

The latencies along this central diencephalic pathway in the ablated series demonstrate that the transmission along it is very slow. A gross estimate of the conduction time from the region of the anterior pole of the superior colliculus to the caudate nucleus,

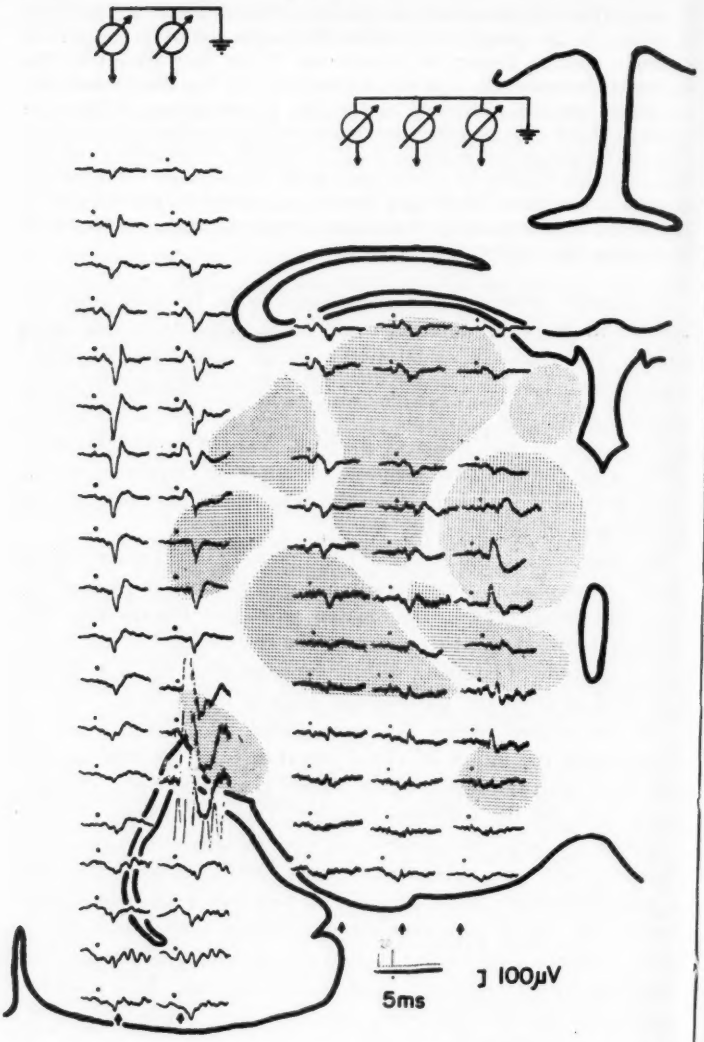

Fig. 1. Topographical plot of subcortical responses to visual stimulation recorded with *monopolar* technique (reference electrode on occipital bone, negative up) in F + 7.5. Arrows indicate extension of needle tracts. Shaded areas indicate main subcortical structures of the plane (see fig. 2). Chronic preparation with ablation of visual cortex bilaterally, 14 days previously. See text.

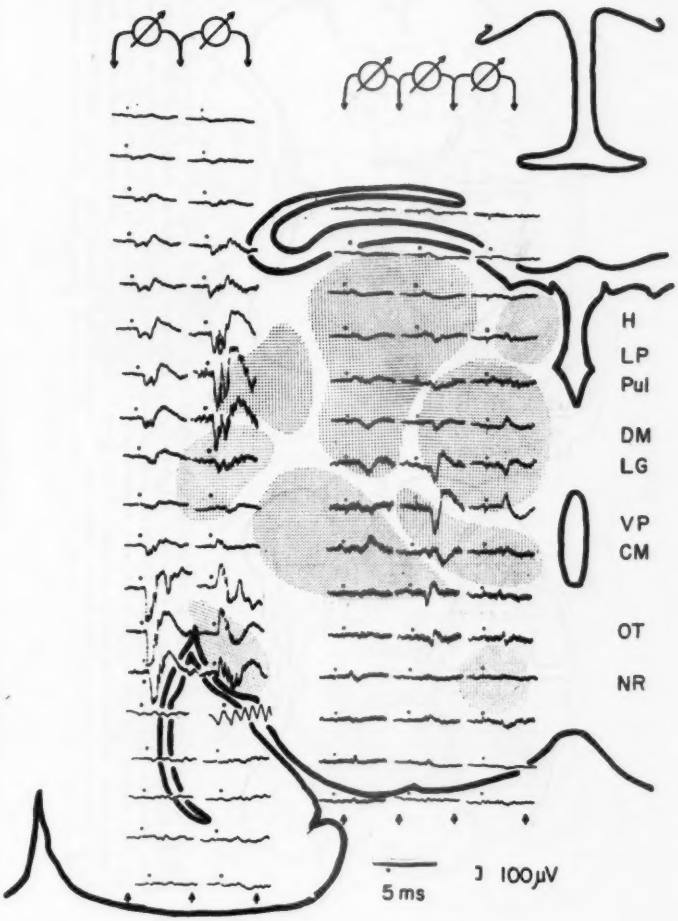

Fig. 2. Responses obtained in *bipolar* recording from the same electrode positions as in fig. 1 in the same experiment. Main structures indicated at the level of their centers. See text and fig. 9 for abbreviations.

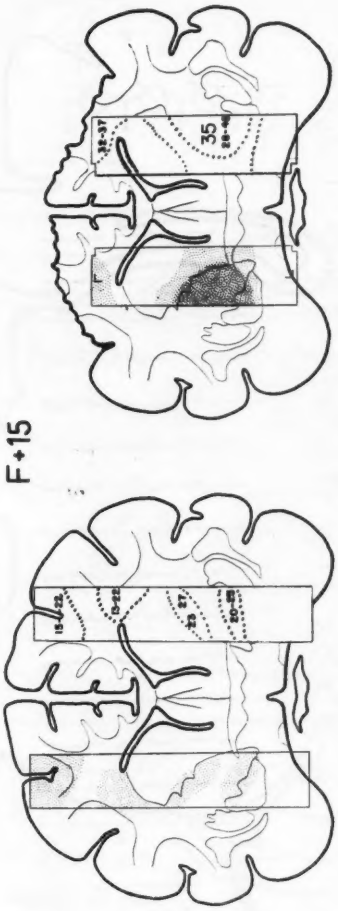

Fig. 3 A.

Fig. 3. Summarizing diagram of results of subcortical explorations in *intact series* (sections on the *left*) and in *ablated series* (sections on the *right*). Explored areas indicated by frames. Number of needle tracts with 4-needle electrode indicated by small angles within frames. Below number of plane is indicated adjacent explored planes which have been approximated to sections represented in figure. *Left hemispheres:* Shaded areas indicate localization and size of responses to light stimulation, arbitrarily divided into small (light grey), medium (grey) and large (dark grey) responses. *Right hemispheres:* Large numbers indicate gross average latency in fields corresponding with those in left hemispheres. Small numbers indicate range of latencies.

a distance of about 10 mm, would give approximately 10 msec., *i. e.*, a velocity of 1 meter per second (see Discussion). These larger responses could be followed to the caudate nucleus, and the longest latencies (37 to 53 msec.) were found in the gyrus cinguli.[1]

[1] Surface cortical explorations in the ablated series demonstrated on some occasions *visual* evoked potentials in the ectosylvian gyrus with a latency of 32 msec.

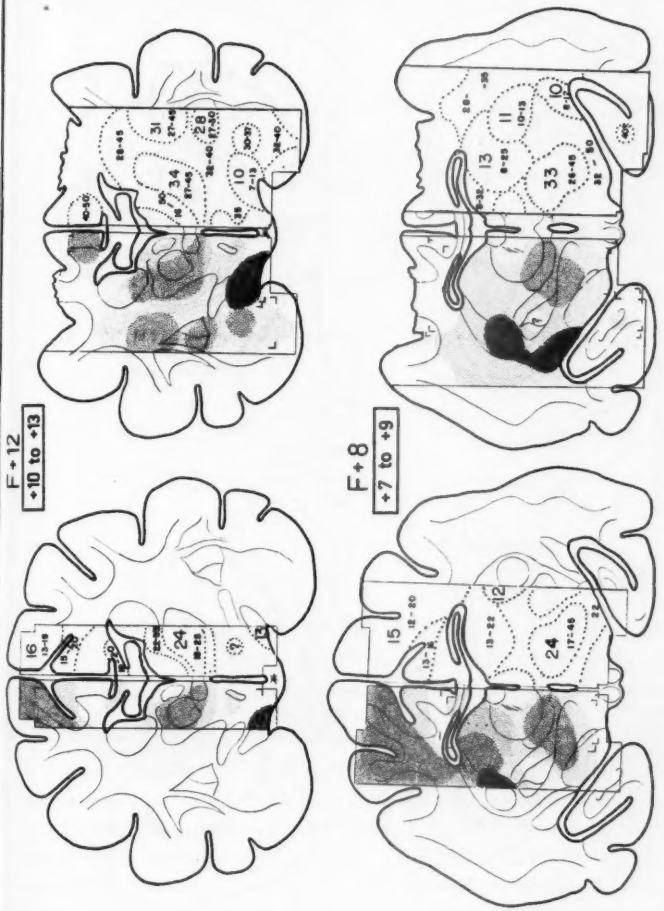

Fig. 3 B.

In the parasagittal, as well as in the frontal charts (fig. 3, right column of brain sections), it is seen that the visual responses in the ablated series also involve other areas, such as the hypothalamus, the basal ganglia and the internal capsule. The explorations did not fully cover mesencephalic structures. It is evident,

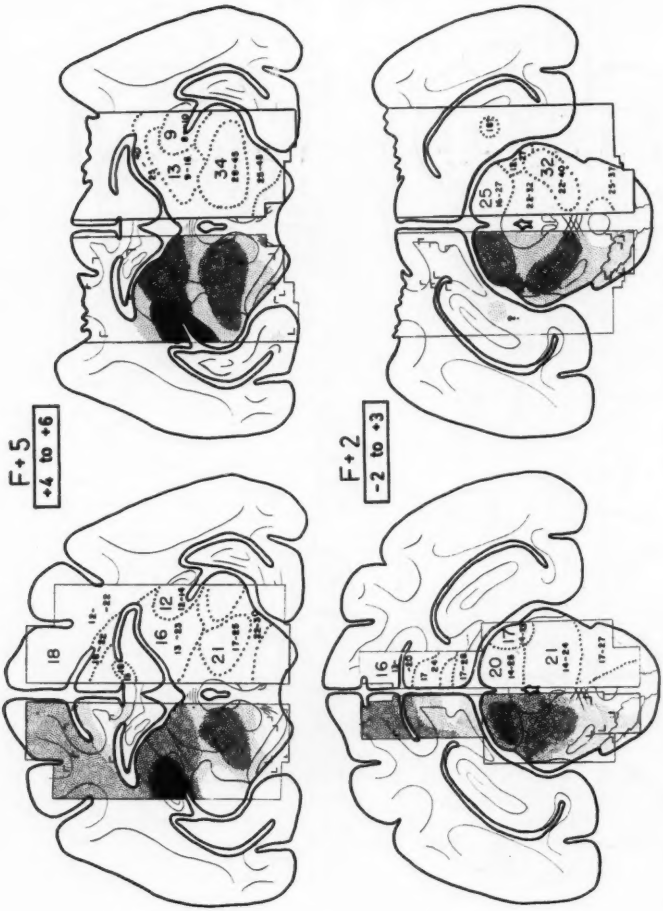

Fig. 3 C.

however (especially in parasagittal plane 6, fig. 8), that large responses with a comparatively short latency may be traced deep into the brain stem at the pretectal level. A number of large responses were also seen in the tegmentum in the mesencephalic reticular formation ventrally to the pretectal area.

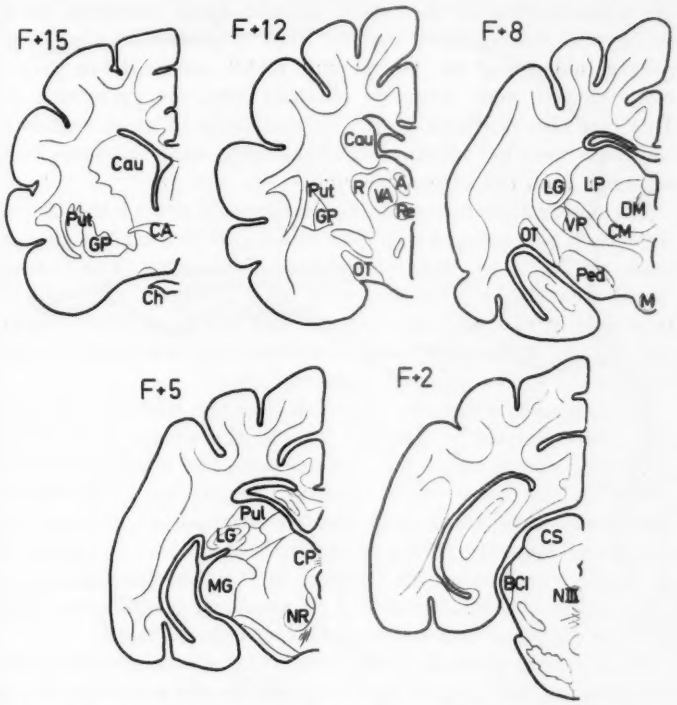

Fig. 4. Main structures of sections in fig. 3. Abbreviations in fig. 9.

In contrast to the findings of the ablated series, those of the *intact series* show that the only defined topographical gradient of latent periods which can be distinguished is the one in the primary optic structures, *i. e.*, from the optic tract, to the lateral geniculate body (as well as around these structures) and to the visual cortex. In the brain stem, the unspecific visual responses are in general later than in the primary optic pathway. There is, however, a rather scattered variation of latent periods within a narrow range (the majority being between 20 to 30 msec.) of these responses. One would therefore not be justified in identifying a distinct central diencephalic pathway for visual impulses in the intact series.

Responses to illumination occurred, of course, all through

the visual cortex of the intact animals. Large responses, indicating well defined fibre bundles, were often seen in the white matter underlying the lateral and middle suprasylvian gyrus. No responses were, however, obtained from the gyrus cinguli. This fact also contrasts to the ablated series in which cingulate responses were not uncommon. Occasionally small responses were recorded from the corpus callosum.

The observations in the intact animals would give a transmission time from the optic tract to the visual cortex from less than 1 msec. up to 8 msec. in various regions of the cortex. This finding would seem to be consistent with other studies of the conduction time in this pathway (BISHOP and O'LEARY 1938; CHANG and KAADA 1950), if it is taken into account that cortical areas outside the striate one were explored also.

It is apparent from the diagrams that the cortical events in the intact animals in general precede the events in the brain stem by several msec. (see Discussion). The latency measurements in the intact series confirm the studies by HUGELIN, BARTHIER, BONVALLET and DELL (1952) and by BONVALLET, DELL and HUGELIN (1952) in unanesthetized cats. The majority of larger visual responses recorded in the diencephalon and in other subcortical structures (*e. g.*, in the basal ganglia), according to their diagrams, had a latency shorter than 30 msec.

A uni-lateral ablation of the visual cortex was carried out in two experiments, one acute and one chronic. In the acute one it was confirmed that the subcortical responses to light stimulation could not be demonstrated in the ablated hemisphere probably due to surgical after-effects (HUNTER and INGVAR 1955). In the chronic one (23 days), however, some visual responses of short latency were found in the intact hemisphere, and some of longer latency in symmetrical regions in the hemisphere with the ablation (fig. 10).

Finally, a control experiment was made in which the temporal lobes were ablated (carefully avoiding the connections between lateral geniculate body and the visual cortex) followed by subcortical exploration of responses to light. In this experiment, visual responses in the thalamus were of the "early" type, *i.e.*, no responses with latencies exceeding 30 msec. were recorded. (Metrazol, injected, later during the experiment, confirmed that the irradiation of visual impulses in this experiment was of the "early" type in frontal cortex (*cf.* HUNTER and INGVAR 1955).) The

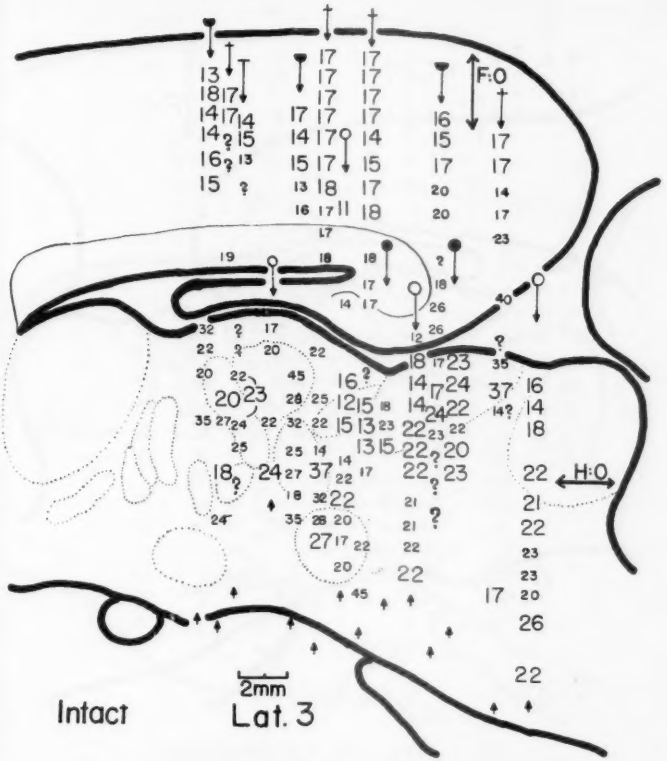

Fig. 5. Parasagittal plot of responses to light stimulation in intact series in a lateral plane 3 mm from midline. Numbers indicate average latency, large numbers represent large and medium sized responses, small numbers represent small responses. Arrows indicate extension of needle tracts. Same symbol on top arrow indicates same experiment. Main subcortical structures of plane indicated by dotted lines, as in fig. 9.

results of this experiment, together with those from experiments with uni-lateral ablation, were therefore consistent with the view that the prolongation of the latencies of visual responses in the brain stem in the ablated series was due to the absence of the visual cortex. This conclusion is also consistent with observations in the experiments with metrazol (HUNTER and INGVAR 1955).

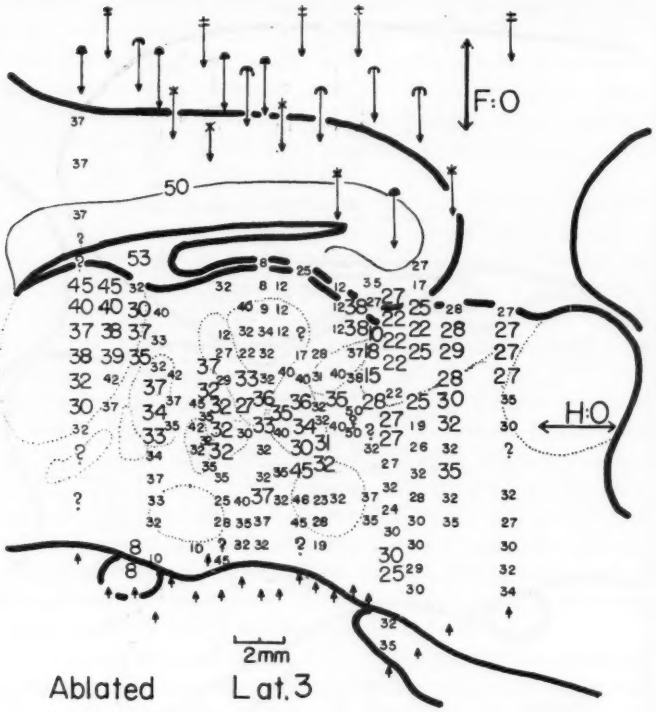

Fig. 6. Same as fig. 5 in ablated series.

In one experiment of the ablated series the cerebellum was removed while visual responses were being recorded from the thalamus. Responses in central thalamic structures could still be obtained after this procedure. This would exclude that the unspecific visual responses in the ablated series necessarily depended upon a cerebellar relay (SNIDER 1952). The observations were not, however, numerous enough to establish topographical differences in the distribution and latency of brain stem visual responses before and after cerebellar ablation. In the same experiment, the superior colliculi were removed bilaterally. After this procedure visual responses could still be recorded in the thalamus (see Discussion).

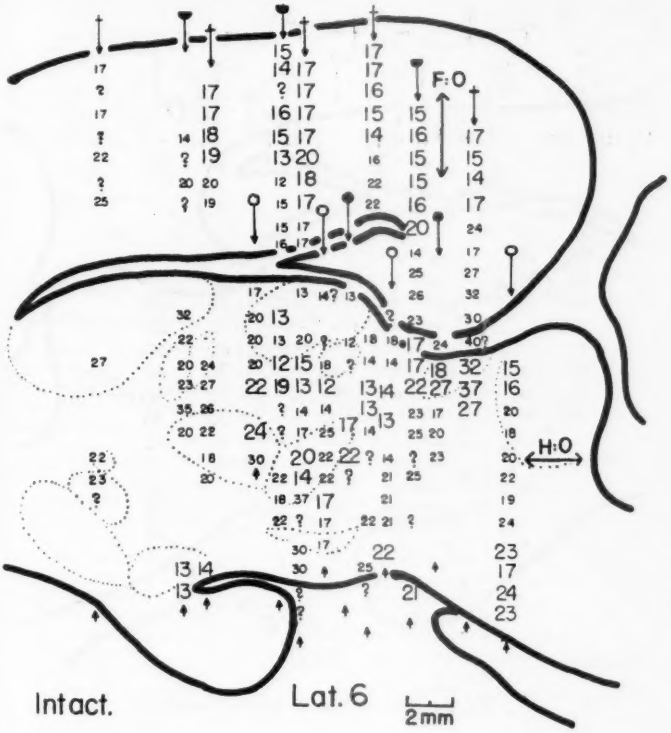

Fig. 7. Parasagittal plot in lateral plane 6. Intact series.

Discussion.

It is not possible to evaluate in details the significance of the differences encountered between the two series, since the full implications of large cortical removals cannot be estimated from the anatomical or functional point of view. There is, however, evidence to support the view that some of the visual impulses, recorded in the brain stem in the ablated series, represent unspecific visual pathways in a "pure" form as they appear when the visual cortex is absent. It has been possible to demonstrate a slow transmission (> 40 msec.) of visual impulses to the frontal cortex under metrazol after *acute* ablation of the visual areas

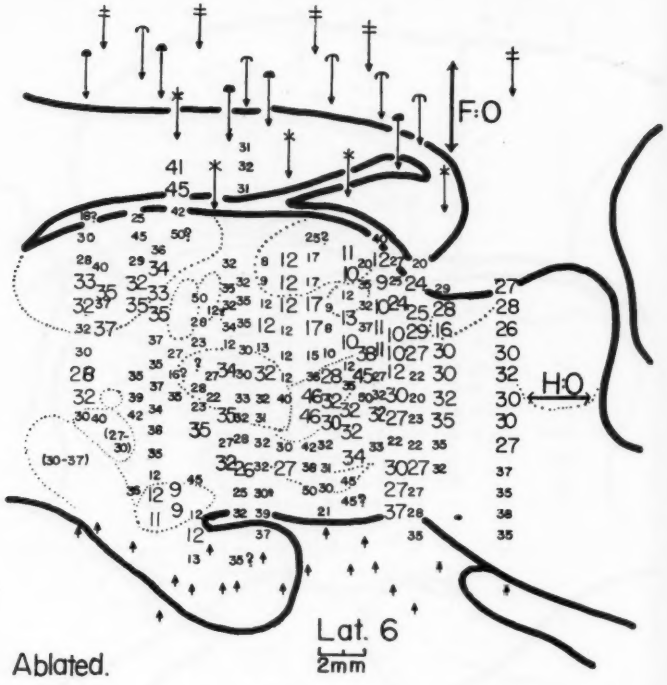

Fig. 8. Same as fig. 7. Ablated series.

(HUNTER and INGVAR 1955). This is not consistent with the possibility that the slow cortico-petal flow centrally in the diencephalon in the ablated series has been caused by degenerative processes. There is also a fairly good agreement in the brain stem latencies between the ablated animals, in spite of the fact that ablation had been carried out at varying periods before the final exploration. The data presented will not, however, give any information as to a "collateral" nature of the visual inflow to the brain stem in the ablated series.

The question might be raised whether the ablated animals demonstrated prolonged brain stem latencies due to pure surgical effects. Such a possibility is contradicted by the fact that, at the time of the final exploration, the ablated animals demonstrated shorter latencies in primary optic structures and, in general, a wider distribution of sub-

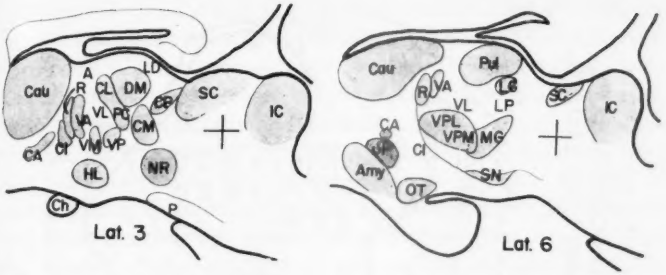

Fig. 9. Main structures of brain stem in parasagittal sections of fig. 5 to 8.

Abbreviations used in the illustrations:

A	anterior nuclei of the thalamus
Amy	n. amygdalae
BCI	brachium colliculi inferioris
CA	anterior commissure
Cau	n. caudatus
Ch	chiasma
CI	internal capsule
CL	n. centralis lateralis
CM	n. centrum medianum
CP	posterior commissure
CS	superior colliculus
DM	n. medialis dorsalis
GC	central gray matter
GP	globus pallidus
GM	medial geniculate body
GL	lateral geniculate body
H	n. habenularis
HL	lateral hypothalamus
LP	n. lateralis posterior
LG	lateral geniculate body
M	mamillary body
MG	medial geniculate body
NCM	n. centralis medialis
NCP	n. commissurae posterioris
NIII	n. of oculomotor nerve
NR	n. ruber
OT	optic tract
P	cerebral peduncle
PC	n. paracentralis
PRT	pretectal area
Pul	Pulvinar
Put	putamen
R	n. reticularis
Re	n. reuniens
SC	superior colliculus
SN	Substantia nigra
VA	n. ventralis anterior
VL	n. ventralis lateralis
VM	n. ventralis medialis
VP (VPL, VPM)	n. ventralis posterior (lateral and medial parts).

cortical responses than did the intact ones. The intact preparations were probably somewhat less active due to the craniotomy immediately preceding the exploration.

Another question to be dealt with is whether pure physical phenomena (see Potential pattern) could have accounted for the difference in latency between the two groups. At present, it does not seem possible to appreciate the influence upon brain stem potentials, recorded with bipolar technique, which might be brought about by the potential field of a mass of synchronously active geniculo-cortical elements. There is, however, one strong evidence against the view that such physical factors would altogether account for the shorter latencies of the brain stem responses in the intact series. In many cases the potentials recorded in unspecific regions of the thalamus, for example, appeared as envelopes of unit spikes (in spite of the size of the needle tips). It must be concluded that such potentials, at least, were generated by neuronal activity close to the needle tips.

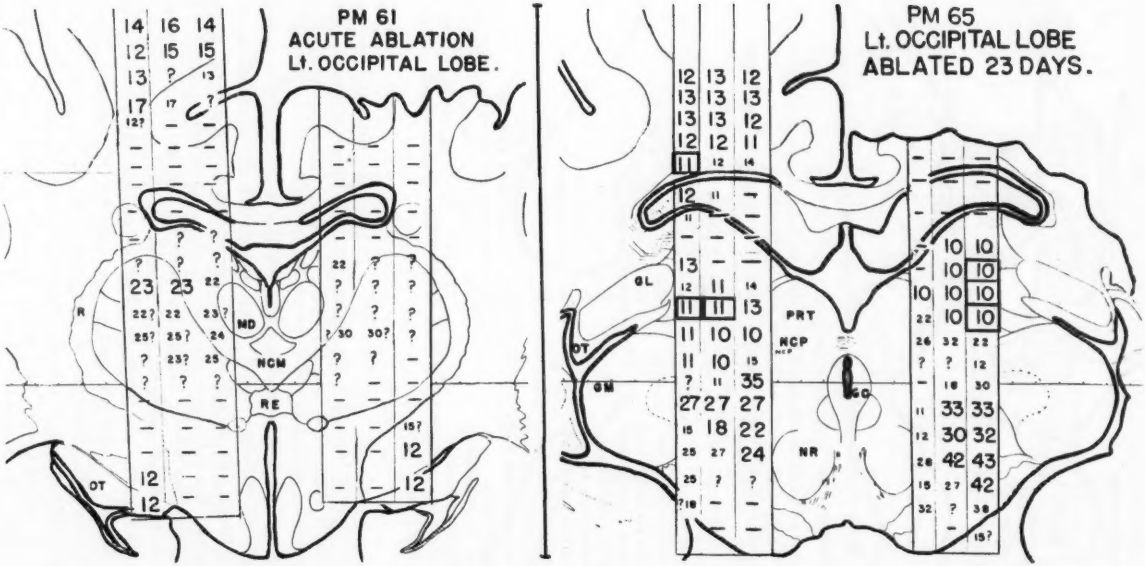

Fig. 10. Brain stem responses to light, recorded in two experiments with uni-lateral ablation of visual cortex. See text. Small figures, small responses. Large figures, medium sized responses. Figures in squares, very large responses.

When the visual cortex is ablated, the major inflow of visual impulses to the brain stem probably takes place (in the cat) through retino-collicular connections (BARRIS, INGRAM and RANSON 1935). Visual impulses may then be propagated from the tectum to various regions caudally and rostrally. In the cat, as well as in other animals, important tecto-spinal and tecto-pontine tracts have been demonstrated. Tecto-thalamic, tecto-incertal as well as tecto-reticular connections are also known (CROSBY and HENDERSON 1948; BUCHER and BÜRGI 1950). BUCHER and BÜRGI (1950) also confirmed the existence of a tecto-thalamic tract passing n. centrum medianum in rostral direction.

However, visual impulses could be demonstrated in the thalamus even after removal of the colliculi in one experiment of the ablated series. This observation confirms the existence of other pathways from primary optic structures to the brain stem than those going through the superior colliculus (MAGOUN and RANSON 1935). WALL, REMOND and DOBSON (1953) studied such pathways in an investigation of the effects of visual impulses upon the motor cortex. These authors also carried out collicular removals and they were able to demonstrate a pretectal inflow of visual impulses which could influence the motor cortex. They conclude that such impulses may be transmitted through the ventral division of the lateral geniculate body (POLYAK 1952).[1] It would also seem that the question must at present be left open of whether there exist other (fine fiber) pathways in the cat from primary optic structures to the pretectal area and the diencephalon (O'LEARY 1940; see also GETZ 1952).

The localization in the diencephalon of what seemed to be the main corticopetal pathway for light impulses in these preparations (fig. 6 and 8) corresponds in general to those structures which have been termed the "diffuse thalamic projection system" or the "thalamic reticular system" (n. centrum medianum, the intralaminar nuclei and some of the nuclei of the rostral pole of the thalamus; MORISON and DEMPSEY 1942; JASPER 1949; STARZL and MAGOUN 1951; STARZL and WHITLOCK 1952; HANBERY and JASPER 1953; HANBERY, AJMONE-MARSAN and DILWORTH 1954). The pathway in question furthermore coincides with anatomically proven fiber systems with rostral direction in the intralaminar parts of the thalamus, recently demonstrated by NAUTA and

Fig. 10. Brain stem responses to light, recorded in two experiments with uni-lateral ablation of visual cortex. See text. Small figures, small responses. Large figures, medium sized responses. Figures in squares, very large responses.

[1] With WALL et al. (1953) we are inclined to question the existence of the accessory optic tracts in the cat (FREY 1951).

WHITLOCK (1954). It is also of interest to point out that visual impulses were recorded (in the ablated series) from the gyrus cinguli. This region of the mesial cortex of the hemisphere has often been demonstrated to have intimate anatomical and physiological connections with the thalamic reticular system and other regions of the reticular formation of the brain stem (ROSE and WOOLSEY 1948; SLOAN and JASPER 1950; KAADA 1951).

The slow conduction velocity of impulses in unspecific pathways of the brain stem — confirmed here in the ablated series for visual impulses — has been interpreted to indicate a polysynaptic organization of such corticopetal systems (MORUZZI and MAGOUN 1949; FRENCH, VERZEANO and MAGOUN 1953 a). In another report (INGVAR and HUNTER 1955) the effects of anesthesia upon brain stem visual responses will be reported. It was found that they were blocked by barbiturates, a fact confirming the investigations of FRENCH, VERZEANO and MAGOUN (1953 b) and of ARDUINI and ARDUINI (1954). The sensitivity to anesthesia and also to surgical procedures on the brain (see above) would be consistent with a polysynaptic organization of the pathways in question. There is also physiological evidence from thalamic stimulation studies to support this view (HANBERY, AJMONE-MARSAN and DILWORTH 1954).

Considering the general differences in brain stem latencies of visual responses, obtained between the intact and the ablated series, mainly one explanation seems to offer itself.

Brain stem responses with the shorter latency in the intact series could, it seems, be explained as due to corticofugal influences from the visual cortex. From the timing of the visual events in the intact preparations it appears possible that impulses from the visual areas would be capable of influencing those events in the brain stem which are initiated by the direct pretecto-tectal visual inflow.

The data do not, however, provide an answer as to whether the slow direct brain stem inflow is "swamped" by the early corticofugal effects in the intact brains, or whether the corticofugal impulses would be able to facilitate conduction along the central diencephalic pathway such as to give the shorter latencies. This question will remain unclarified until more is known about the detailed organization of the unspecific afferent pathways medially in the brain stem and critical interaction data might be obtained.

Since the cortical ablations comprised both the striate and the peri- and parastriate areas (visual areas I and II) no information is obtained from the results presented as to the relative importance of the various parts of the visual cortex for the effects observed following a complete visual cortical removal. From existing anatomical and physiological evidence, it would seem, however, that the cortical areas surrounding the striate area are capable of a more wide-spread subcortical influence than the striate cortex is itself (POLYAK 1927; NIEMER and JIMENEZ-CASTELLANOS 1950; JASPER, AJMONE-MARSAN and STOLL 1952). VON MONAKOW (1895), POLYAK (1927) and BARRIS, INGRAM and RANSON (1935), have demonstrated corticofugal connections from the striate area in the cat to the tectal region. Such fibers are also coming from the peri- and para-striate areas and some of these continue to the pontine nuclei. From the occipito-parieto-temporal cortex there are also connections to the thalamus, the hypothalamus, the subthalamic region and the tegmentum (NIEMER and JIMENEZ-CASTELLANOS 1950; JASPER, AJMONE-MARSAN and STOLL 1952).

The findings thus suggest that (in the cat) the visual cortex might exert a control upon other events in the central nervous system elicited by optic stimulation. From a more general point of view such a supposition may have wider implications. The development of central visual mechanisms provides one of the best examples of "encephalization" of function which is taking place in the phylogenetic scale (cf. KAPPERS, HUBER and CROSBY 1936 and MARQUIS 1934). The present results would suggest that the younger geniculo-cortical system is capable of controlling visual functions pertaining to phylogenetically older parts of the brain stem. Applying this view to more recent concepts, it would seem that the specific visual systems through their cortical projection areas and the corticofugal fiber systems are able to control events in the unspecific pathways of the brain stem. Such a principle, if confirmed in other animals with other modalities, would add measurably to the understanding of the functional organization of the afferent pathways of the central nervous system.

Summary.

1. Stereotaxic explorations have been carried out in the brain stem of unanesthetized cats of the topography and latencies of responses to optic stimulation. In one series intact preparations

have been used and in a second group animals with chronic bilateral ablation of the visual cortex.

2. In both the intact and the ablated preparations, visual impulses were found to have a wide-spread distribution in the brain stem, a fact which, in principle, confirms the observations of others by the fact that afferent responses in "unspecific" regions are not unconditionally dependent upon the cortical projection area of the modality tested.

3. In the ablated preparations it was possible to trace a central diencephalic pathway for light responses which coincides topographically with the thalamic reticular system as well as with recently demonstrated fiber systems in intralaminar parts of the thalamus. The latencies of responses along this pathway which was followed to the gyrus cinguli indicated a slow conduction velocity. This pathway could not be distinguished in the intact preparations.

4. The latencies of the visual brain stem responses in the intact preparations were generally shorter than those in the ablated series. This observation has been interpreted as an effect of corticofugal influences from the visual cortex upon the brain stem.

5. The results suggest that the visual cortex (in the cat) is capable of controlling events elicited by visual stimulation in other (phylogenetically older) parts of specific visual structures as well as in unspecific visual pathways of the brain stem.

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Über spezielle Charakteristika afferenter vagaler Herznervenimpulse und ihre Beziehungen zur Herzdynamik.

Von

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Eingegangen am 18. Oktober 1954.

In einer früheren Arbeit haben NEIL und ZOTTERMAN (1950) A-Faserentladungen vom Herzen beschrieben, die rhythmische Aktivität mit den Änderungen des Venendruckes zeigten. Inhalt der vorliegenden Arbeit soll eine genauere Differenzierung dieser A-Faserpotentiale auf verschiedene Variationen des Druckes und ihre Beziehung zur Hämodynamik sein. ADRIAN und ZOTTERMAN (1926) haben mit steigender Reizintensität eine Zunahme der Impulsfrequenz für die Druck-Receptoren im Skelettmuskel gefunden. Ein analoges Verhalten des Herzmuskels wurde von ZOTTERMAN (1948) vermutet, und wir glauben, dies nachweisen zu können. Freilich gelingt am Herzen eine Einzelfaserpräparation nicht mehr. Doch konnten wir unter geeigneten Bedingungen eine sichere Beziehung zwischen Venendruck und Impulsfrequenz für zweierlei A-Fasern nachweisen. Wir werden sie, — entsprechend den Arbeiten von MATTHEWS (1933) am Skelettmuskel, als A_1- und A_2-Fasern bezeichnen. Ihr Verhalten wurde unter den verschiedensten Versuchsbedingungen beobachtet.

Technik und Methodik.

Die Untersuchungen der vagalen Herzafferenzen wurden an 40 Katzen, in der von JARISCH und ZOTTERMAN (1948) beschriebenen Weise durchgeführt. Durch exakte Zeitbestimmungen zu den einzelnen Herzrevolutionen und Zuordnung der Impulse zum simultan registrierten EKG und fortlaufender Venendruckschreibung nach WETTERER (1943),

WETTERER und PIEPER (1952), versuchten wir zu einer sinnvollen Analyse dieser oft verwirrend vielfältigen Bilder zu gelangen. Die Relation zu den einzelnen Phasen der Herzdynamik ist dabei wesentlich aufschlussreicher als etwa die zum EKG. Hier zeigten sich die besonderen Vorzüge der fortlaufenden Venendruckregistrierung mit der manometrischen Sonde nach dem Prinzip des Differentialtransformators nach WETTERER (1943), WETTERER und PIEPER (1952). Die Sonde hat einen äusseren Durchmesser von 2.5 mm. Die Einführung in die Vena brachialis erfolgt daher auch bei der Katze beinahe mühelos. Der Ausgang des Verstärkers ist an den 2-Strahlenoszillographen angepasst. Die Empfindlichkeit kann stufen*los* zwischen 0 und dem Höchstwert verändert werden. Der druckaufnehmende Teil befindet sich an dem vorderen Ende des Katheters, sodass sich keine Flüssigkeitssäule zwischen der Messtelle und dem Manometer, ausbreitet; die Eigenfrequenz beträgt etwa 600 Hz.

Das operative Vorgehen ist in den vorhergehenden Arbeiten von ZOTTERMAN (1948, 1950) ausreichend dargestellt. Wichtig erscheint uns die Konstanterhaltung der Körpertemperatur des Versuchstieres, sowie des Luftgemisches bei der künstlichen Atmung. (Bei CO_2-Überlagerung ist die Zunahme der Receptorenaktivität im Lautsprecher und Aktionsstrombild deutlich erkennbar.) Die Methodik der Ableitungen ist in den vorhergenannten Arbeiten ebenfalls festgelegt. Um Änderungen im Aktionsstrombild und dem EKG zu vermeiden, darf das Tier nur *ein*mal geerdet werden (SCHÄFER, 1950).

Ergebnisse.

Die von ZOTTERMAN (1948) und von NEIL und ZOTTERMAN (1950) beschriebenen A-Faserimpulse in den afferenten Herznerven, synchron den Venendruckänderungen, die der P- und T-Zacke im EKG entsprechen, werden durch unsere Versuche bestätigt. (S. Abb. 1.)

Abb. 1 ist ein charakteristisches Beispiel für alle unsere Kurven, aus denen immer wieder ein deutlicher Anstieg in der Entladungsfrequenz parallel mit dem Venendruckanstieg hervorgeht. Der Abfall des Venendruckes liess dagegen keine besondere A-Faseraktivität erkennen. In einer besonders geglückten Ableitung konnten einige Details dieser A-Impulse festgestellt werden. (Abb. 2, a—c.)

Abb. 2 a zeigt bereits eine Verlagerung der präsystolischen Vorhofswelle in die Systole. Sonst entspricht diese erste Impulswelle genau dem Venendruck*anstieg*, der unter gewöhnlichen Bedingungen mit der P-Zacke des EKG zusammenfällt. In Abb. 2 b findet sich eine deutliche Frequenzzunahme der A_1-Potentiale (1) und der A_2-Potentiale (x). Für diese Impulssalven ist allein der

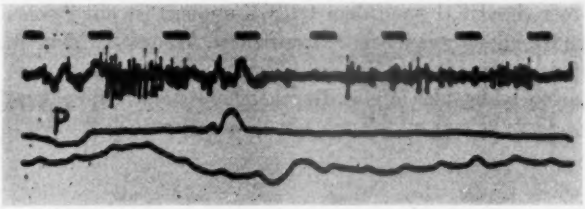

Abb. 1. Oben: Impulse verschiedener A-Fasern, deren zeitliche Zuordnung zu den Venendruckänderungen sichtbar ist; dazwischen: EKG. Beachte die genauen Zeitbeziehungen zw. EKG u. Venendruck (VD) anstieg, besonders nach P! Darunter: VD-registrierung (50 Hz-brumm = techn. Störung). Zeit: 1/25 Sek.

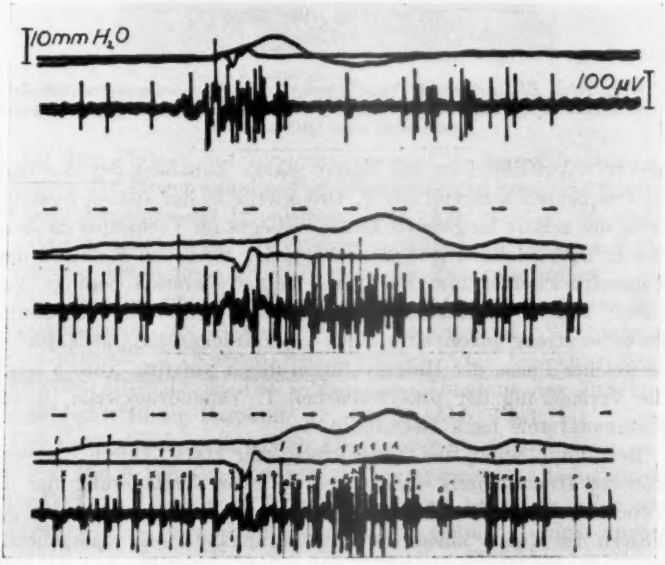

Abb. 2. Von oben nach unten: Venendruck, EKG, Impulse des oberen Herzvagusastes (Katze).

a. VD-anstieg bis zu einem Maxim. von 9 cm H_2O. Mitte bis unten:
b. VD-Max. 13.5 cm H_2O. Ausbildung von 3 syst. VD-wellen. Veränderung der zeitlichen Relation zum EKG. Frequenzzunahme der A_1 (1) u. A_2 (x)-Potentiale.
c. VD-Max. 16.5 cm H_2O weitere Frequenzzunahme der A-Impulse.

Erratum in Abb. 2: 10 mm H_2O soll 6 cm H_2O sein.

Venendruckanstieg (von 9.0 Max. bis 16.5 cm H_2O in Abb. 2 c) massgebend. Das Verschwinden der präsystolischen 1. Druckwelle und die Verlagerung der Venendruckwelle in die Systole der

Kammer (im EKG nach dem QRS-Komplex) ist ein Zeichen beginnender hämodynamischer Insuffizienz. Wenn das Herz stärker insuffizient wird und Störungen des venösen Rückstromes auftreten, so bildet sich die 1. Druckwelle zu einer doppelgipfligen Dromedarwelle aus und auch die 2. Druckwelle tritt deutlicher

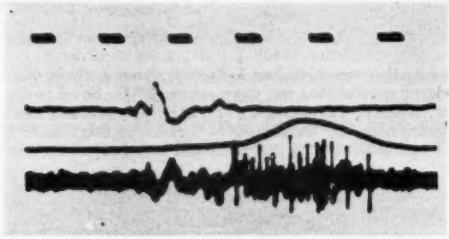

Abb. 3. Oben: EKG, darunter Venendruck. A-Faserimpulse entsprechend dem Venendruckanstieg nach 10 mg Belladoninsulfat i.v. Verschiebung der V-Druckwelle nach dem QRS-komplex.

hervor. Auffallend ist die relativ starke Zunahme der A_1- und A_2-Frequenzen während der 2. Druckwelle in der späten Systole, trotz des relativ langsamen Druckanstieges im Verhältnis zu dem der 1. Druckwelle. Die Veränderung der zeitlichen Relation der Venendruckwellen zum EKG sind hämodynamisch bedingt. Sie haben keinen Einfluss auf die Impulsaktivität der A-Fasern. Diese ist streng gekoppelt an den Venendruckanstieg, gleichgültig in welcher Phase der Herzrevolution dieser auftritt. Abb. 3 zeigt die Verlagerung der präsystolischen 1. Venendruckwelle in die Kammersystole nach Belladonin.[1]

Belladonin senkt den Venendruck sehr stark. Durch die veränderte Herzdynamik kommt es zu einer Verlagerung der 1. Venendruckwelle in die Systole. Für die Impulssalven der A-Fasern ist daher allein die Venendruckänderung massgebend. Diese direkte Beziehung zwischen der Impulsfrequenz und dem Venendruckanstieg in Abb. 2 a—c lässt sich zahlenmässig festhalten:

	Venendruckanstieg bis zu einem Maximum in cm H_2O	Entladungsfrequenz der A_1-Receptoren	Entladungsfrequenz der A_2-Receptoren
a.	9.0 cm	14	41
b.	13.5 cm	31	48
c.	16.5 cm	29	63

[1] Der Firma E. Merck-A. G., Darmstadt, sei für die Überlassung von Versuchsmustern gedankt.

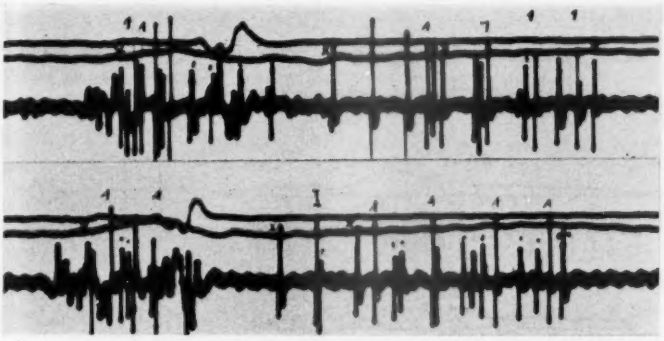

Abb. 4.

a. Impulssalven bei *arteriellem* Mitteldruck von 110 mm Hg. $f_1 = 6$ (A_1-Pot. (1). $f_2 = 3$ (A_2-Pot. (x). (.) = Faseraktivität.
b. Impulssalven bei art. Mitteldruck von 50 mm Hg. (1 Min. nach 10 mg Parpanit i.v.) $f_1 = 6$. $f_2 = 3$.

Auf dem Plateau des Venendruckes sind die Impulsfrequenzen geringer. Bei länger anhaltendem Druck erkennt man eine relativ schnelle Abnahme der Impulsfrequenzen (Abb. 2 a—c), die wohl für eine Adaptation der Receptoren spricht. Sie scheinen auf die Änderung des venösen Druckes nach der Zeit empfindlich.

Die Höhe des *arteriellen* Mitteldruckes ist für das Verhalten der Impulsfrequenz der A-Fasern nicht massgebend. Wir zeigen vergleichsweise 2 Kurven bei normalem und bei sehr tiefem arteriellen Mitteldruck. Die schlagartige Abnahme des Druckes wurde durch 10 mg Parpanit[1] i.v. erzielt. (Abb. 4 a u. b.)

Der Venendruck ist in beiden Ableitungen annähernd gleich geblieben. Die A-Impulse, die eine so ausgesprochene Abhängigkeit ihrer Frequenze von *venösen Druckänderungen* aufweisen, scheinen auf *arterielle* — bei gleichbleibendem Venendruck — nicht anzusprechen. Die rapide Senkung des arteriellen Mitteldruckes ohne jeden äusseren, mechanischen Eingriff gelang durch Parpanit (10 mg i.v.).

Eine besonders interessante Frequenzanalyse gelang in Abb. 5 a—c. Zur einfacheren Übersicht ist die besonders typische Phase des Venendruckanstieges bis zum QRS-Komplex, welche etwa der Zeit der Vorhofskontraktion entsprechen dürfte, in schematischer Skizzierung neben dem Aktionsstrombild festgehalten.

[1] Wir danken der Firma Geigy-A. G., Basel, für die Überlassung von Versuchsmustern.

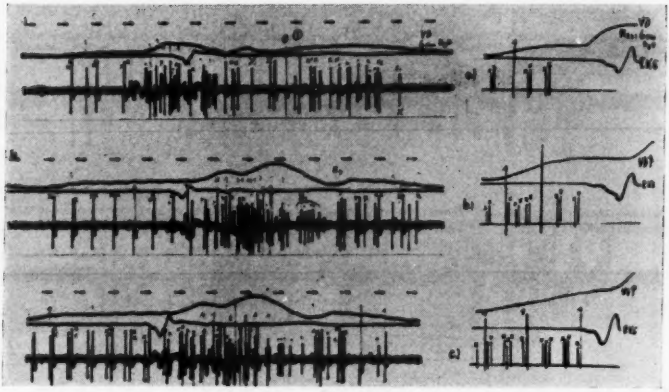

Abb. 5. Aktionsstrombild u. schematische Skizzierung der A_1- und A_2-Impulse während fortlaufenden Venendruckanstieges. Beachte Frequenz und Gruppierung!

a) 3 Dublées A_2 (x). 1 A_1 (1.) (Vd-Max.: 6 cm H_2O.)
b) 5 Dublées A_2 (x). 1 Dublée A_1A_2. (VD-Max.: 13.5 cm.)
c) 3 Triplets A_2. 1 Triplet A_1A_2. 2 Dublées A_1A_2. 1 A_2 (bei weiteren VD-anstieg VD-max.: 16.5 cm H_2O).

In Abb. 5 wird die Abhängigkeit der Frequenz vom Venendruckanstieg nochmals im Detail illustriert. Während in Abb. 5 a 3 Dublées A_2 und 1 A_1-Entladung im Bereich des aufsteigenden Venendruckbogens zu sehen sind, findet man bei steigendem Venendruck in 5 b bereits 5 Dublées A_2 und 1 Dublée A_1A_2.

Bei weiter steigendem Venendruck treten in demselben Intervall 3 Triplets A_2 und 1 Triplet A_1A_2, 2 Dublées A_1A_2 und 1 A_2-Impuls auf. Die zunehmende Frequenz der Entladungen lässt die in Abb. 5 a noch beinahe verdeckten — da zeitlich ineinanderfallenden Dublées von A_2-Impulsen in Abb. 5 b deutlich hervortreten. Bei weiterem Druckanstieg geht die Dubléebildung in einer Tripletbildung über, wobei die Desynchronisation durch Änderung der Impulsfrequenz eine Analyse des Aktionsstrombildes erleichtert.

In diesen Abbildungen 5 a—c, sowie in Abb. 2 und 4 findet sich neben den A-Impulsen auch eine kleinfasrige Aktivität, die wir, der Einteilung Erlangers und Gassers folgend, als δ-Fasern bezeichnen wollen, sie erlauben analog den A-Fasern eine Einteilung in δ_1, und δ_2 Potentiale, während die grössten gemessenen A-Potentiale dieser Präparation eine Höhe von 20—21 mm zeigen, haben die B-Faserpotentiale nur 7 bzw. 11 mm Ampli-

tude. Das Verhältnis der Potentialhöhen dieser Fasern, wenn die höchsten A-Fasern (I = vermutlich Mediastinal-Receptoren) = 100 % gesetzt werden, ergibt folgende Tabelle:

	Spike-höhe in mm	Potential d. Faseraktionsstromes in Prozent des Pot. der dicksten Faser.
I.	20 mm	100 %
A_1	14.5 mm	72.5 %
A_2	13.0 mm	65 %
δ_1	11.0 mm	55 %
δ_2	7.0 mm	35 %

Die δ-Faseraktivität tritt nur in extremen Fällen besonders deutlich hervor, da sie meist von der A-Faseraktivität überlagert und verdeckt wird. Besonders deutlich war sie, wenn eine besonders starke *Dehnung* des Vorhofes schon durch Betrachtung des Herzens festzustellen war. Der Dehnung ging ein starker Druckanstieg des Maximal- und Minimaldruckes voran.

Die Bestimmung der Frequenzen für die δ_1 und δ_2-Fasern ergibt:

	Venendruckanstieg bis zu einem Maximum in cm H_2O	f δ_1	f δ_2
	7.5 cm	1	3
Abb. 2 a	9.0 cm	7	1
» 2 b	13.5 cm	21	3
» 2 c	16.5 cm	15	6

Ein anschauliches Bild der δ-Faseraktivität, der deutlichen Incisur vor Einsetzen der Vorhofsdruckwelle und der entsprechenden A-Faseraktivität zeigt Abb. 6.

Erst bei höheren Graden der Vorhofsdruckzunahme, wenn eine vermehrte *Dehnung* der schwachen Vorhofswände sichtbar wird, tritt die δ-Faseraktivität hervor. Wir vermuten daher, dass diese Receptoren vorwiegend auf Volumenänderungen ansprechen: die δ_1-Receptoren mehr auf Dehnung, die δ_2-Receptoren mehr auf Entdehnung. Maximale Vorhofsdehnung bei völliger Arrhythmie nach experimentellen Coronarinfarkt lässt auch bei relativ niedreren Venendrucken diese δ-Faseraktivität vermehrt hervortreten. (Zotterman und Struppler, 1954.) Es können jedoch diese Ergebnisse noch nicht als abgeschlossen betrachtet werden und bedürfen weiterer Nachprüfung.

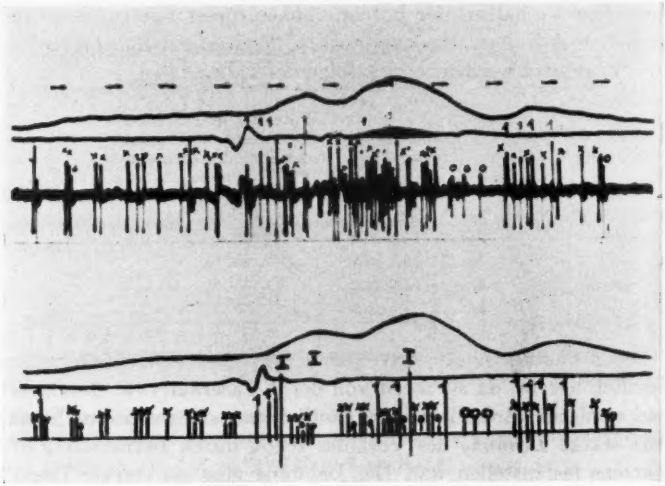

Abb. 6. Zunehmende δ-Faseraktivität bei weiter ansteigendem Venendruck nach Abb. 5. Vorhofswand*dehnung*.

b) Schematische Darstellung von Abb. 6. A_1 (1) 1 Impulse. A_2 (x) 2 Impulse. I: Besonders grosse A-Impulse (Mediastinal-Receptoren?).

Diskussion.

Das Problem der Herzmuskel-Receptoren liegt in ihrer *räumlichen* und *zeitlichen Summation*, sodass oft nur extreme Versuchsbedingungen eine Analyse des komplexen Aktionsstrombildes ermöglichen. Neben der Verkleinerung des receptiven Feldes durch möglichst weitgehende Aufsplitterung des Nerven — die jedoch in 10 mm Entfernung vom schlagenden Herzen nur sehr begrenzt ist — konnte durch verschiedene Variationen physikalischer Reize eine Verbesserung der Resultate erzielt werden. Doch sind diesen Bemühungen relativ enge Grenzen gesetzt, wenn man bedenkt, dass in den afferenten Vagusfasern etwa 20 × so viele Impulse zentralwärts laufen als effektorisch zum Herzen zurückkehren (SCHÄFER, 1950). Die Rückmeldung in den afferenten Herznerven über die Arbeitsleistung, wie sie R. WAGNER (1950) angenommen hatte, wurde experimentell bestätigt (STRUPPLER, 1954, im Druck).

Zunächst fällt auf, dass alle diese Impulse, nicht wie etwa er-

wartet — eine Abhängigkeit von der Kammer, sondern allein eine deutliche Beziehung zur Tätigkeit der Vorhöfe haben. Es scheint sich daher um Receptoren aus den Vorhöfen zu handeln, die histologisch von NONIDEZ (1937) sichergestellt wurden. Der *adäquate Reiz* für die von AMANN und SCHÄFER (1943), JARISCH und ZOTTERMAN (1948), NEIL und ZOTTERMAN (1950) beschriebenen A-Fasern ist die Venen*druckänderung*. Da die *Volum*änderungen der Vorhöfe, während der ersten und zweiten Venendruckwelle *gegensinnig* verlaufen, die Impulssalven der A-Fasern jedoch in beiden Fällen auftreten, so kann man annehmen, dass es sich um Druck- oder Spannungs-Receptoren handelt. Bezüglich des Volumens würde beim 1. Druckanstieg eine Verminderung desselben eintreten, da der Vorhof sich in den Ventrikel entleert, während bei der 2. kleineren und langsamer ansteigenden Druckwelle, das Volumen zunimmt, da sich der Vorhof von den Venen her füllt. Ferner ist bei der Volumenzunahme während der Erschlaffung der Vorhöfe, jedoch bei gleichzeitigem Druck*abfall* keine erhöhte Impulsaktivität zu sehen. Es erscheint daher berechtigt, die verantwortlichen A-Receptoren als *Druck*- oder *Spannungs*empfindlich und nicht als Volumenabhängig zu bezeichnen.

Eine rein isometrische Phase, d. h. gleichbleibendes Volumen bei verändertem Druck, gibt es beim Vorhof, der kein geschlossenes System darstellt, überhaupt nicht. Die Versuchsbedingungen so zu variieren, dass dies weitgehend möglich wird, soll Gegenstand weiterer Arbeiten sein. Der Skelettmuskel, dessen Spannungsempfindlichkeit unter rein isometrischen Bedingungen untersucht werden kann, stellt natürlich den Idealfall dar, der am Herzen nie erreicht werden kann. Es scheinen jedoch weitgehend ähnliche Gesetze für den Herzmuskel zu gelten. Diese A-Receptoren des Herzens müssen sich daher so verhalten, als lägen sie in Serie geschaltet mit der Muskelfaser, wie dies von WHITTERIDGE (1948) für die venösen A-Fasern bei seiner Ableitung vom Halsvagus erkannt wurde (WAGNER, 1950, STRUPPLER 1954). Aus allen unseren Kurven geht deutlich hervor, dass der Venendruck*anstieg*, der adäquate Reiz für diese A-Faserentladung ist, während beim Abfall keine besondere Faseraktivität erkennbar ist. Auch Änderungen des *arteriellen* Druckes (Abb. 4) scheinen keine Erregung dieser Receptoren hervorzurufen. Man kann dagegen einwenden, dass etwa eine sehr kurzdauernde arterielle Druckänderung und die entsprechenden Salven unserer Beobachtung

entgangen sein könnten und diese Receptoren eben auch nur auf die Änderungen nach der Zeit ansprechen. Dies lässt sich nicht sicher ausschliessen, da solche Versuche nicht angestellt werden konnten. Auf konstanten Druck sprechen auch die venösen A-Fasern nicht an, sie sind also für die absolute Höhe nicht empfindlich. Die Abhängigkeit der Impulzfrequenz von den Druckänderungen geht aus Abb. 2, 5 und besonders 6 deutlich vervor. ZOTTERMAN (1950) hat die Vermutung ausgesprochen, dass es sich bei diesen A-Receptoren des Vorhofes um Druck- oder Spannungs-Receptoren handeln müsse, wie er sie bereits 1926 mit ADRIAN (ADRIAN und ZOTTERMAN, 1926) für den Skelettmuskel beschrieben hat, handeln müsse. Für die Spannungs-Receptoren des Vorhofes glauben wir dieselbe Gesetzmässigkeit gefunden zu haben. Sie sind auf den Differentialquotienten des Druckes nach der Zeit empfindlich, also $\frac{dp}{dt}$. Dieses Verhalten zeigt Abb. 2, 5 und 6 a—c. Während länger anhaltenden Druckes erkennt man eine relativ schnelle Abnahme der Impulsfrequenz, die wir als *Adaptation* dieser A-Receptoren deuten wollen. Dieses Verhalten stimmt mit den Versuchen von ADRIAN (1928) überein, der die grösste Adaptationsgeschwindigkeit für markhaltige *Druck*-Receptoren bei konstanter Reizung im Gegensatz zu den sehr gering adaptierten Muskel-Receptoren beschrieb. Für die auffallende, relativ starke Frequenzzunahme während des langsameren Venendruckanstieges in der 2. Druckwelle können wir vorläufig keine Erklärung geben.

Ein anschauliches Bild von der Parallelität der Impulsfrequenz und dem Druckanstieg vermittelt Abb. 5. Die Gruppierung der A-Potentiale verändert sich während des Venendruckanstieges infolge der Frequenzzunahme. Ob diese Zunahme in demselben Intervall durch einfache Steigerung der Frequenz ein und derselben Receptoren oder durch Einschaltung neuer A-Receptoren hervorgerufen wurde, lässt sich nicht mit Sicherheit entscheiden. Der Desynchronisation nach, welche sich in Abb. 5 b und c herausbildet, dürfte es sich wohl um letzteres handeln.

JARISCH und ZOTTERMAN haben bereits 1948 nachgewiesen, dass die A-Faseraktivität nach Drosselung der venösen Zufuhr durch Abklemmen beider Vv. Cavae völlig verschwindet und erst nach Freigabe der Zirkulation wiederkehrt.

Einige mehr theoretische Beobachtungen scheinen uns für die Beurteilung der Versuchsergebnisse noch von Bedeutung: respira-

torische Schwankungen durch Zug an den Vorhöfen sind meist durch äussere Ursachen bedingt und können in vielen Fällen vermieden werden. Jeder Zug an den Lungenwurzeln kann daher atemsynchrone Impulse auch in den Herznerven auslösen. NONIDEZ (1937) hat an den Lungenvenen eine grosse Anzahl sensibler Endorgane beschrieben. Auch atembedingte Füllungsschwankungen der Vorhöfe können entsprechende Periodik der Herznervenimpulse auslösen. Die veränderte Lungenfüllung kann respiratorische Vorhofsdruckänderungen und dadurch Aussendung von A-Impulsen bewirken. Geringe Seitenverschiedenheiten der Impulse vom rechten und linken Vorhof beruhen auf Seitenverschiedenheiten der Vorhofsfüllung rechts und links während der Atmung. Auch die Kammer kann in ihrer Systole durch Tiefertreten der Ventilebene rein mechanisch bedingte Venendruckänderungen hervorrufen, die sich in einer kammersynchronen Entladung äussern, wie sie SCHAEFER (1950) beschrieb. Diese Faktoren müssen besonders unter extremen Versuchsbedingungen beachtet werden, da sie sich nicht immer völlig eliminieren lassen.

Während sich für die venösen A-Fasern eine sichere Beziehung ihrer Impulsfrequenz zum Druck*anstieg* in der Zeiteinheit nachweisen lässt, ist dies für die δ-Fasern und ihre Beziehung zu den *Volumen*änderungen wesentlich schwieriger, da eine fortlaufende Volumenkontrolle des Herzens in situ nicht möglich war. Ein direkter Nachweis, dass diese Receptoren nur auf Änderungen des Volumens ansprechen und eine genauere Frequenzanalyse im Verhältnis zu den Volumenänderungen ist *uns* bis heute noch nicht möglich gewesen. Wir können uns daher nur auf indirekte Folgerungen, wie wir sie für die Beurteilung der Druck- oder Spannungs-Rc. (A-Rc.) im Vergleich zur Volumenänderung durchführten, beziehen. Während sich gerade im Beginne des Venendruck*anstieges* eine deutliche Frequenzzunahme der A-Rc. in allen Versuchen findet, die mit Erreichung des Venendruckmaximums — häufig sogar schon vor dem Kulminationspunkt — sistiert, zeigt sich die δ-Aktivität erst nach längerem Druckanstieg und auch beim Abfall des Venendruckes. Dass es sich jedoch bei diesen δ-Afferenzen nicht einfach um druckaufnehmende Receptoren höherer Reizschwelle handelt, zeigt folgende Beobachtung: unter extravaganter hämodynamischer Bedingung, etwa bei völliger Arrhythmie mit massiver Vorhofsüberdehnung nach experim. Coronarinfarkt (STRUPPLER, 1954) findet sich auch bei *ab*sinkendem Venendruck, diese δ-Aktivität deutlich vermehrt, während die A-

Faseraktivität beinahe völlig verschwindet. Sicherlich haben diese wesentlich dünneren δ-Fasern eine höhere Reizschwelle als die A-Fasern des Herzens. Diese δ-Impulse dürften mit den von WHITTERIDGE und PAINTAL (1953) beschriebenen venösen B-Fasern identisch sein. Wir möchten jedoch an der Einteilung von ERLANGER und GASSER festhalten und die Bezeichnung B-Fasern den autonomen präganglionären Fasern vorbehalten.

Es scheint, dass diese beiden Typen der Herzafferenz aus den Vorhöfen der Katze, wie schon WAGNER (1950) postulierte, dem Zentralnervensystem die Rückmeldung über Druck- und Volumenänderungen, d. h. über seine Arbeitsleistung vermitteln (STRUPPLER, 1954).

Zusammenfassung.

Der adäquate Reiz für die Druck- oder Spannungs-Receptoren der Vorhöfe am Katzenherzen (A-Receptoren analog dem Skelettmuskel) ist die ansteigende Venendruckänderung nach der Zeit. Auf die absolute Höhe sprechen sie dagegen nicht an. Die Abhängigkeit der Impulzfrequenz vom Venendruckanstieg ist nahezu linear, die Adaptationsgeschwindigkeit ist relativ hoch. Die A-Receptoren sind daher auf den Differentialquotienten des Druckes nach der Zeit empfindlich. Auf Änderungen des art. Druckes sprechen sie unter den üblichen experimentellen Bedingungen nicht an. Sie verhalten sich wie in Serie zum kontraktilen Element geschaltet.

Unter extremen hämodynamischen Bedingungen lässt sich spontan eine kleinfasrige δ-Aktivität hervorrufen. Diese Fasern scheinen vorwiegend auf *Volumen*änderungen, d. h. Änderungen der Vorhofswanddehnung anzusprechen. (δ_1 auf Zunahme der Wanddehnung, δ_2 auf *Ent*dehnung.)

Zeitliche und räumliche Summation der verschiedenen Herz-Receptoren sind das Kernproblem afferenter Vagusimpulse, die das Zentralnervensystem über Druck- und Volumenänderungen in den Vorhöfen orientieren.

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Investigations into the Euglena Gracilis Method for Quantitative Assay of Vitamin B_{12}.

By

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Received 22 October 1954.

Since, in 1949, HUTNER, PROVASOLI, HOFFMANN, BELT, FRANKLIN, STOKSTAD and JUKES described the use of the alga *Euglena gracilis* var. *bacillaris* for the quantitative assay of vitamin B_{12}, this method and modifications of it have been widely used. *Euglena gracilis* has the advantage over the various lactobacilli also used for this purpose that desoxyribonucleosides cannot replace B_{12} as a growth factor for this organism. Besides, the Euglena method is generally said to be more sensitive than are the lactobacillus methods. HOFF-JØRGENSEN (1954), by using *Lactobacillus Leichmannii* (ATCC 7830) in an improved medium, however, obtained about the same sensitivity with the two methods.

The following experiments indicate that the sensitivity of the Euglena method and the shape of the growth curves depend on the age of the Euglena culture used for inoculation, its dilution, and whether the cells are washed or not. The influence of these conditions can be related to the finding reported in this paper that a thermolabile substance, which inhibits the growth of Euglena, is formed in the medium during the growth of the cells.

Experiments.

The medium used was that described by ROSS (1952).

Stock cultures of Euglena were maintained by weekly subculture in test-tubes (13 × 155 mm) containing 10 ml of single-strength basal medium with 80 $\mu\mu$g of vitamin B_{12} per ml.

Inoculum: 3 to 8-day-old stock cultures. The cultures were washed as follows: 5 ml of the culture was centrifuged aseptically. The cells were washed twice with 5 ml portions of sterile single-strength medium and resuspended in the same medium. Dilutions of the cultures were also made with the sterile single-strength medium.

Incubation: 10 days at 30° C in a perspex water thermostat illuminated by fluorescent strip lights.

Standard curve: A standard solution containing 100 $\mu\mu$g of vitamin B_{12} per ml was prepared from a stock solution containing 10 μg of B_{12} per ml. The standard solution was pipetted in triplicate to test-tubes (16 × 100 mm) in the following amounts: 0.0 — 0.1 — 0.2 — 0.3 — 0.4 — 0.6 and 1.0 ml. The volumes were made up to 2 ml with water, and 2 ml of double-strength basal medium were added to give a final volume of 4 ml. The final concentrations of B_{12} were thus: 0 — 2.5 — 5— 7.5 — 10 — 15 and 25 $\mu\mu$g per ml. The tubes were plugged with cotton-wool, steamed for 15 minutes and inoculated with 0.05 ml of a culture of Euglena. After incubation growth was determined turbidimetrically in a cuvette using a photo-electric colorimeter (Lumetron 402-E) with a red filter.

Experiment 1: Four sets of standards were inoculated with 0.05 ml of a 6-day-old culture of Euglena containing about 2×10^6 cells per ml:

a. inoculum unwashed, undiluted
b. » » , diluted 1 + 6
c. » washed, undiluted
d. » » , diluted 1 + 6.

Experiment 2: Four sets of standards were inoculated with 2 cultures of different age:

a. An 8-day-old culture containing about 2.5×10^6 cells per ml, unwashed, diluted 1 + 7.
b. The same culture as in "a" in the same dilution, but washed.
c. A 3-day-old culture containing about 0.9×10^6 cells per ml, unwashed, diluted 1 + 2.
d. The same culture as in "c" in the same dilution, but washed.

Experiment 3 A: A 7-day-old stock culture of Euglena containing about 2.5×10^6 cells per ml was centrifuged aseptically. Varying amounts of the supernatant were added in triplicate to test-tubes containing 4 ml of single-strength basal medium with 12.5 $\mu\mu$g of B_{12} per ml:

a. 0.2 ml of supernatant, undiluted, *i. e.* a final concentration of 50 μl/ml.
b. 0.2 ml of supernatant, diluted 1 + 9, *i. e.* a final concentration of 5 μl/ml.
c. 0.2 ml of supernatant, diluted 1 + 99, *i. e.* a final concentration of 0.5 μl/ml.
d. No supernatant, 0.2 ml of single-strength basal medium.

The tubes were steamed for 15 minutes and inoculated with 0.05 ml of a washed 4-day-old culture diluted 1 + 3. Growth was determined turbidimetrically after incubation for 10 days.

Experiment 3 B: Same as 3a with the exception that the sterile supernatant in this experiment was added after steaming of the test-tubes *i. e.* the supernatant was not steamed.

Experimental Results.

Experiment 1: Fig. 1 shows that the undiluted, unwashed 6-day-old culture (curve a) does not grow nearly as well as when diluted (curve b). The *washed*, undiluted culture (curve c) grows much better than the corresponding culture *unwashed* and un-

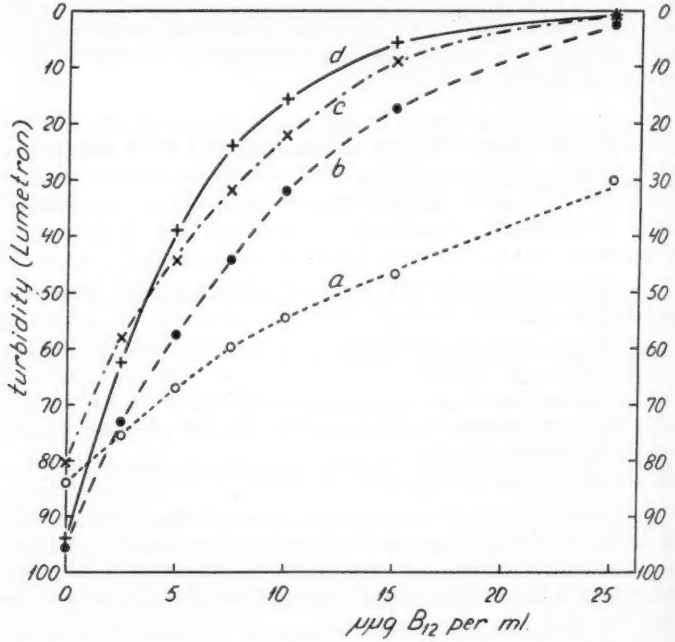

Fig. 1. Growth curves for *Euglena gracilis.*

Inoculum: A 6-day-old Euglena culture containing about 2×10^6 cells per ml:
curve a, inoculum unwashed, undiluted
» b, » » , diluted 1 + 6
» c, » washed, undiluted
» d, » » , diluted 1 + 6
Incubated for 10 days at 30° C, in the light.

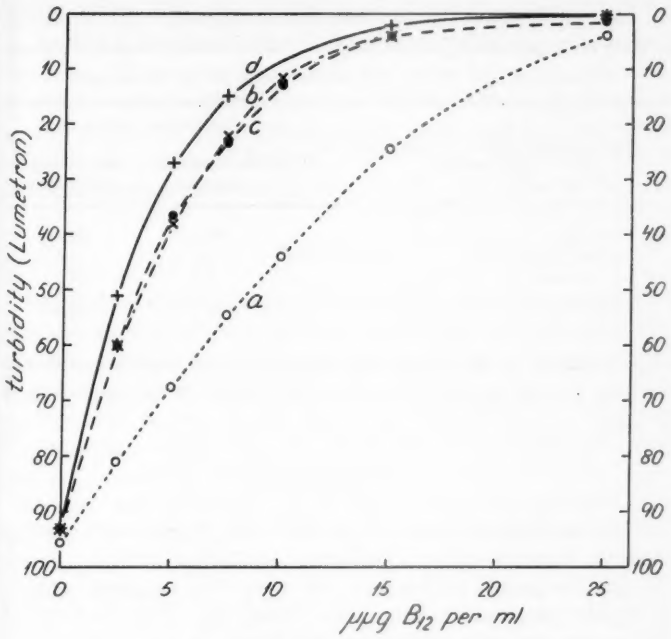

Fig. 2. Growth curves for *Euglena gracilis:*

curve a, inoculum: An 8-day-old culture containing about 2.5×10^6 cells per ml, unwashed, diluted 1 + 7.
» b, the same culture as in "a", in the same dilution, but washed.
» c, inoculum: A 3-day-old culture containing about 0.9×10^6 cells per ml, unwashed, diluted 1 + 2.
» d, the same culture as in "c", in the same dilution, but washed.
Incubated for 10 days at 30° C, in the light.

diluted. The best growth is obtained by inoculation with a washed and diluted culture (curve d).

Experiment 2: Fig. 2 shows that the 3-day-old unwashed and diluted culture (curve c) grows better than the unwashed and diluted 8-day-old culture (curve a); but when washed, both cultures of different age give almost the same growth (curves b and d).

Experiments 3 A and 3 B: Table I shows that the sterile supernatant from a 7-day-old centrifuged culture of Euglena exerts a marked growth-inhibiting effect when added *after* steaming. The inhibitory effect first appears when the final concentration of supernatant is about 0.5 μl per ml and is almost complete at a

Table I.

Effect of the supernatant from an Euglena culture on the growth of Euglena.

12.5 $\mu\mu$g B_{12} per ml, 10 days incubation at 30° C in the light.

final concentration of supernatant in $\mu\mu$g per ml	turbidity (Lumetron)	
	supernatant steamed	supernatant unsteamed
0 (no B_{12})	90.5	90.5
0 (12.5 $\mu\mu$g B_{12}/ml)	6.5	6.5
0.5 (.....)	7.5	12.5
5.0 (.....)	7.0	46.0
50.0 (.....)	6.5	88.5

concentration of 50 μl per ml. When steamed together with the medium for 15 minutes the inhibitory effect of the supernatant is lost.

Discussion.

The above experiments show that the supernatant from a growing Euglena culture contains a thermolabile factor, which inhibits the growth of Euglena cells when they are transferred to a freshly prepared medium containing B_{12}. This finding has apparently not previously been described.

It is strange that a 7-day-old culture can present a marked density (about 2.5×10^6 cells per ml) in spite of the presence of the growth-inhibiting factor. The explanation may possibly be that the inhibitory factor primarily influences the B_{12}-absorption of the cells, which normally takes place very rapidly (according to Ross, 1952, 50 $\mu\mu$g of B_{12} per ml will have been absorbed in the course of 48 hours). During the first few days following inoculation the amount of inhibitory factor formed is small, if present at all. The cells may therefore at this time be able to absorb the vitamin B_{12} — and store it intracellularly. Their continued growth can then take place in spite of the inhibitory factor formed later.

Whether the inhibitory factor directly influences the Euglena cells or the vitamin, *e. g.* by the formation of a new compound, which is not utilized by the Euglena, does not appear from the present study. Further experiments are necessary to elucidate whether its formation is more directly associated with the growth of the Euglena cells, regardless of the composition of the medium.

The preliminary experiments presented above have lead the

author to the following modification of the Euglena method for quantitative determination of B_{12}: For establishing a standard curve the following range of final concentrations of B_{12} are used: 0 — 1.25 — 2.5 — 3.75 — 6.25 — 8.75 and 12.5 $\mu\mu$g per ml in a total volume of 4 ml. Inoculation is made with 0.05 ml of a 3 to 8-day-old culture of Euglena, washed twice with sterile single-strength medium and diluted to about 3×10^6 cells per ml.

Summary.

Using Ross's modification of Hutner's method for quantitative determination of vitamin B_{12} with *Euglena gracilis* var. *bacillaris*, experiments were carried out which showed that the supernatant from a centrifuged vigorously growing Euglena culture contains a thermolabile factor, which has a growth-inhibiting effect on Euglena cells when they are transferred to a freshly prepared medium containing vitamin B_{12}.

Density and uniformity of growth in the tubes inoculated are increased by washing and diluting the Euglena culture used for inoculation. The sensitivity and the accuracy of the method are thereby increased.

Acknowledgement.

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Effect of Irradiation with X-Rays on the Catabolism of Ethylalcohol in the Mouse.

By

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Watery suspensions of numerous enzymes can promptly be inactivated, often by exposure to a restricted dose of ionizing radiation, as first shown by DALE (1942). This type of prompt inactivation of enzymes is not often met in vivo, possibly due to the presence of a great variety of protecting substances and a rapid whipping out of possible changes produced by exposure. FORSSBERG for example (1945) did not succeed in inactivating catalase present in the liver even when he applied as massive doses as 83,000 r. In those experiments, the splitting effect of liver slices of controls and exposed animals on hydrogen peroxide was compared and in the study of the effect of irradiation on other enzymes their effect on other suitable substrates.

Enzyme inactivation can also be studied in the intact animal by administering a metabolite marked with ^{14}C and studying the amount of labelled CO_2 exhaled previous to and after exposure. If irradiation reduces the amount of labelled CO_2 exhaled this does not prove an inactivation of the enzymes involved; viz. the effect of exposure may be due to other reasons than enzyme inactivation and so far the latter took place, it may be difficult to ascertain which of the enzymes involved in the catabolic process was influenced. If, however, irradiation does not effect the amount of exhaled CO_2 we can conclude that the enzyme system

involved in the catabolic process was not influenced by exposure or, more correctly, that a sufficient fraction of enzymes involved remained intact and can thus perform its task at a normal rate. In an earlier communication (1953) we found mice previously exposed to a dose of 1,500 r to catabolise methylalcohol at the same rate as controls. In the present note the results of a study of the effect of exposure of mice to irradiation with X-rays on the catabolism of ethylalcohol are communicated.

Experimental.

Albino mice, weighing 12—24 g, were placed each in one of six perforated aluminium boxes which were fixed in a plexy glass box of 1.5 liter volume. CO_2-free air passed through the box carrying the exhalatory CO_2 which was absorbed by a set of centrifuge tubes containing saturated $Ba(OH)_2$. 3 bottles each containing 50 ml solution proved to be sufficient to absorb all CO_2. The centrifuged $BaCO_3$ precipitate was washed with CO_2-free water and ethanol, dried first in a vacuum exsiccator, then by heating to 105°.

To each mouse 0.050 to 0.100 ml of a 20 p. c. ethyl alcohol solution labelled by addition of 1 to 3 μg alcohol-1-C^{14} were administered by intraperitoneal injection. The exhalatory $^{14}CO_2$ of the same group of mice was collected at intervals of 10 min. first, previous to irradiation and again after the lapse of two days and also after exposure to a dose of 1,500 r. In both cases ethanol was injected in each experiment before the collection of the exhaled CO_2 had started, and within $^1/_2$ hr of the termination of irradiation which took 30 min. The roentgen tube applied was run with 180 kV and 7 milliamp. In some of our experiments the controls were injected in intervals of 2 days. This was made possible by the fact that the specific activity of the exhaled CO_2 declined already after the lapse of 5 hrs. to less than 3 p. c. of its maximum value observed in an early stage of the experiment.

In contrast to the controls exposed mice could only be applied once. Before starting the irradiation food but not water was withdrawn from the mice and from the controls at a corresponding time. In other experiments, food was withdrawn 20 hrs. before starting the experiment, as the food consumed and resorbed by the exposed mice differs from that of controls; even by pair-feeding this source of error cannot be completely eliminated. The experiments were carried out in a thermostat room kept at a constant temperature of 27.1°. The control mice were kept in the same wooden box in which irradiation took place as were the exposed mice. As the amount of CO_2 exhaled shows a diurnal variation the experiments had to be carried out always at the same hour.

The specific activity of the exhaled CO_2 was determined by comparing the activity of $BaCO_3$ samples of identical weight (100 mg) with that of the ethanol injected after converting it into $BaCO_3$.

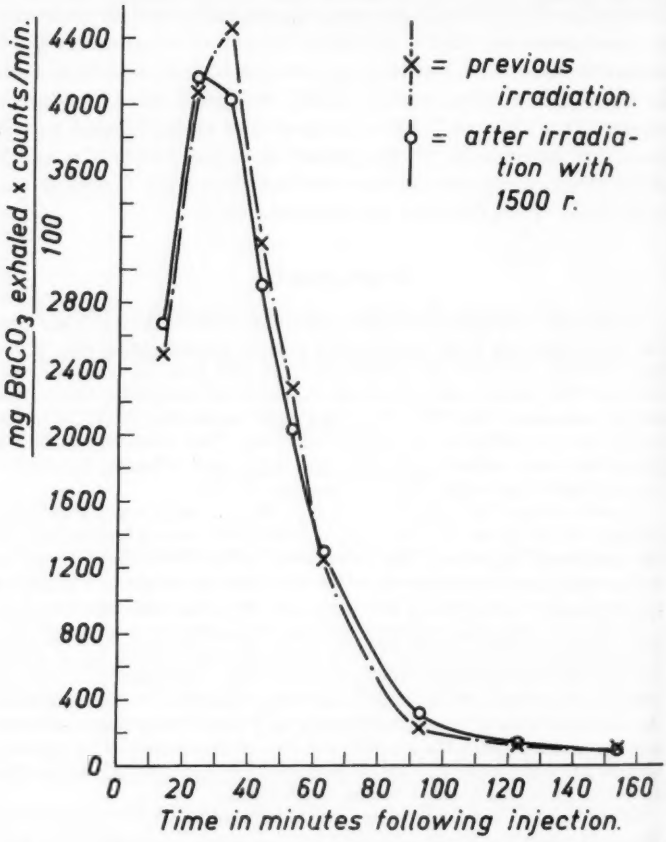

Fig. 1. Effect of irradiation on the exhalation of $C^{14}O_2$ by labelled ethyl alcohol injected mice.

Results.

The result of a typical experiment in which after 20 hrs. of fasting 6 mice were injected with labelled ethanol, whereupon food was given for about a day and the animals irradiated and injected again the second day after 20 hrs. of fasting is seen in Fig. 1. The figures were obtained by plotting the product of the

weight of $BaCO_3$ obtained by collecting the exhalatory CO_2 in baryte, and the activity of 100 mg of that compound. As seen from the figure no significant difference is shown by the amount of $C^{14}O_2$ exhaled by the mice previous to and after irradiation. In 4 further experiments the total amount of $C^{14}O_2$ exhaled by the exposed animals differed by + 14, — 8, — 7, + 12 p. c. from that given off before irradiation. When injecting the same group of mice 3 times without and the fourth time after exposing them to 1,500 r we obtained the following relative figures: 100, 112, 109, 102 for the amount of $C^{14}O_2$ collected.

VITALE and assoc. (1953) investigated the rate of alcohol metabolism in rats administered varying doses of alcohol by using ethanol-1-C^{14} and measuring the $C^{14}O_2$ excreted in the respired air. They found the rate of alcohol oxidation to be directly proportional to the dose administered up to a maximum of about 2.5 to 3 g per kilo of body weight. When 0.9 g alcohol/kg body weight was injected to each rat they found the maximum rate of oxidation of ethanol to amount to 220 mg/kg/hr. during the 2nd and 3rd hour after its administration. We found in our experiments with mice a corresponding value of 197 mg/kg/hr. This result was obtained with animals fed till the start of the experiment. When mice that had been fasting for 20 hrs. were investigated the corresponding figure was 149 mg/kg/hr.

That fasting animals catabolise ethanol at an appreciably lower rate than fed ones was already observed by VITALE and assoc. While they recovered 70—90 % of the alcohol administered to fed rats, the recovery from fasting rats amounted to 35—93 % only. BARTLETT and BARNET (1949, 1953) observed a recovery of 75 % from fed rat in the course of 5 hrs (cf. also DONTCHEFF (1950) and BURBRIDGE and HINE (1951)). Fasting was also found to decrease the ability of liver slices to oxidize ethanol (VITALE et al. 1953). Ethanol was found most rapidly oxidized in such experiments, when its concentration in the surrounding medium was 25 mg% (MASORO et al. 1953).

Summary.

The rate of oxidation of ethylalcohol by control mice and such exposed to a dose of 1,500 r of roentgen rays was compared by administering with alcohol-1-C^{14} labelled ethanol and collecting the $C^{14}O_2$ exhaled. No significant difference was found in the

amount of $C^{14}O_2$ exhaled by the controls and the irradiated animals.

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The Electrocardiogram of Rabbits in Blast Injury.

By

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Statements as to the ECG changes in blast injury are few in the clinical literature and only a few experimental papers deal with the electrocardiographic manifestations of the effects of high explosive shock waves on the cardiovascular system.

Barrow and Rhoads (1944) in a study on 200 cases of uncomplicated blast injury in man state that the main changes are a low voltage and flattened QRS complexes. A moderate bradycardia was found in the majority of their cases but no other form of arrhythmia was observed. Among 48 patients with blast injury examined by Leavell (1945) 5 previously normal men showed evidence of acute congestive heart failure and 3 others had significant changes in ECG, chiefly manifested as alterations of T waves and QRS complexes. As the changes were of only short duration they were interpreted not to be the result of structural changes in the heart such as hemorrhage or infarction but probably due to a circulatory shock.

In one patient Desaga (1950) found an isoelectric take-off of the S—T segments in all leads and pronounced T waves 10 hours after a blast exposure. The most striking change was a widening,

elevation and notching of the P wave, that was interpreted as due to the overstrained pulmonary circulation. The electrocardiographic changes did not subside immediately after the disappearance of the pulmonary hemorrhages. As a rule they further increased, instead, throughout the period of convalescence.

LIAN, FERRABOUC and PÉRIER (1952), finally, found in three patients, during the war exposed to a mine or bomb explosion, auricular fibrillation in two of the cases and negative T waves in V_6 in the third one.

KROHN, WHITTERIDGE and ZUCKERMAN (1942) were apparently the first to record ECG in experimentally produced blast injuries in rabbits. Their chief aim was to find out if animals killed outright by blast die from ventricular fibrillation. The ECG of animals exposed to blast pressures of the order of the P_{50} level (*i. e.* the peak pressure causing lethal injuries to 50 per cent of the animals exposed) only showed occasional alterations in the size of the QRS complexes and ventricular extrasystoles. A slight reduction of the heart rate occurred in some animals. In animals exposed to instantaneously lethal pressures (two or three times the P_{50} level) a reduction of the heart rate by 60 to 70 per cent was invariably noticed. There was also a reduction of the amplitude of the complexes but no change in the coordination of the beats. The bradycardia caused by the detonation was seen also in vagotomized and atropinized animals.

BENZINGER (1950) found in blasted animals an often pronounced bradycardia, ventricular extrasystoles, a high take-off of the T wave, and long P—R interval as a sign of a hypoxic myocardium and sometimes higher degrees of atrioventricular blockage. As the signs of myocardiac hypoxia in the ECG need some minutes to develop this change could be differentiated from the more rare fatal *commotio cordis* where the changes occur immediately after the exposure. In many cases the changes in ECG were correlated with occurrence of an air embolism, and in one experiment, by recompressing the animal to 3 atmospheres in a recompression chamber, the high take-off of the T wave disappeared and the ECG became normal.

CLEMEDSON (1949) in an extensive study on air blast injury recorded ECG in a great number of rabbits. The main findings besides an often very pronounced bradycardia were a sinus arrhythmia sometimes reaching as far as to complete irregularisation of the rhythm, extrasystoles of different origin and different

types of heart block. Ventricular flutter or fibrillation was observed but were uncommon findings. After violent detonations severe changes were seen in ECG indicating serious myocardial lesions, that could be verified by microscopic examination.

LEVINE (1942) described marked spontaneous changes in the normal rabbit ECG and emphasized that the knowledge of these spontaneous changes is essential to the intelligent appraisal of experimental studies on the rabbit heart. The normal rabbit ECG is also thoroughly examined by MASSMAN and OPITZ (1954).

In the previous paper by CLEMEDSON (1949) no detailed accounts of all the different changes in the blast injury ECG were given and only the changes occurring immediately and during the first few minutes after the detonation were dealt with. The scope of this paper is to give a more comprehensive analysis of the various ECG changes in the blast injured rabbit and to follow the development and reversal of the changes during several days. In a part of the material special attention is paid to the spontaneous changes in several ECG before the detonation. Such changes are compared with those obtained after the exposure.

Material and Methods.

The material comprises a great number of ECG obtained from 194 rabbits exposed to a high explosive shock wave. 174 animals were anesthetized, before the exposure, with urethane in a dose of 1.5 g/kg body weight given intravenously in a 20 per cent water solution. The remaining 20 animals were exposed without anesthesia.

The material is divided into two groups. The 62 animals in the first group were exposed to a detonating charge of TNT in the open field. The second group, 132 animals, was exposed in a detonation chamber specially constructed for blast injury studies.

As a quantitative measurement of the extent of the injury the pulmonary hemorrhages are used. The degree of lung hemorrhage is expressed by a quotient $\frac{W_1}{W_0}$, where W_1 is the weight of the blast damaged lung determined at autopsy and W_0 the calculated normal weight of the same lung. A more detailed description of methods is given by CLEMEDSON (1949).

In the open field blastings the weight of charge varied between 0.23 and 1.15 kg and the distance between the animal and the charge was between 0.6 m and 3.0 m.

In the experiments in the detonation chamber a plastic high explosive was used, the chief constituent of which is pentaerythrit tetranitrate (PETN). The weight of charge varied from 2 to 7 g. With 4

to 7 g of charge high peak pressure and impulse values giving severe blast injury were obtained.

A four channel electrocardiograph (Triplex, system Elmqvist) was employed for the ECG recording. In most cases the three standard limb leads were recorded. As electrodes hypodermic needles with a length of 4 cm were employed. They were inserted under the skin and firmly fixed with adhesive plaster round the leg.

In 15 animals repeated ECG were recorded before and after the exposure of the detonation in order to catch spontaneous day-to-day variations in the normal ECG (LEVINE 1942) and to follow up the changes caused by the blast injury. In this group a five-lead ECG was

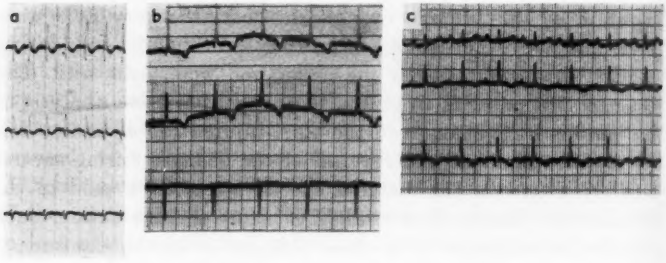

Fig. 1. Standard limb leads in three rabbits showing pathological patterns before the detonation.

recorded with two chest leads in addition to the standard limb leads. The chest region of these animals was clipped and depilated and surface electrodes were applied and fixed with adhesive plaster. A thin layer of gauze soaked in saline was placed under the electrodes to ensure good contact against the skin. The chest leads were taken from the left and right side of the anterior surface of the chest according to LEVINE (1942) with the indifferent (needle) electrode on the left hind leg. The leads are called L and R respectively. The oval-shaped chest electrodes had a contact area of about 18 cm^2 and were placed with their long axis reaching from the apex of the axilla to the midline of the sternum. In these experiments recordings were taken at the following times *viz.*: one every day three to five days before the exposure, immediately before, under and immediately after the detonation and then 1, 2, 15, and 60 min., 3, 6 and 24 hours and 2, 4 and 7 days after the exposure. In the recordings made immediately after the detonation only the chest leads were used, in all other recordings both limb and chest leads were taken. These animals were anesthetized only during the day of exposure.

In most experiments the animals were exposed in a sitting posture in narrow wire mesh cages. Only in a few experiments for special reasons they were exposed and recordings were taken with the animals lying in a recumbent position.

The Electrocardiogram of the Normal Rabbit.

The ECG of 194 apparently normal rabbits were examined before the detonation, 179 only once, 15 several times during 3—5 days. The results of the normal ECG correspond with those found by LEVINE (1942) and MASSMAN and OPITZ (1954). The figures of the normal ECG given in these papers are used at the judgment of the ECG in the present material. In the repeated ECG of one and the same animal some changes were found, as also emphasized by LEVINE (1942). The effect of the detonation on the ECG is more safely recognized in this group of 15 rabbits as the variability of the ECG in the individual rabbit can be regarded. In the present material no spontaneous changes in the rhythm except a slight sinus arrhythmia were observed. Marked changes in the voltage of the QRS complexes and the direction of the T waves in some leads from day-to-day were frequently observed, presumably due to different position of the heart within the thorax although the experimental procedure was unchanged. It must, however, be stressed that the T wave in lead II was found positive in this material as also in LEVINE's (1942) with the exception of 3 animals. These 3 rabbits showed abnormal ECG before the blast injury, according to the above-mentioned criteria (Fig. 1) and with diphasic or negative T waves in lead II as abnormal manifestations among others.

Electrocardiographic Changes Due to Blast Injury.

Only changes certainly pathologic as judged from the predetonation ECG and the figures of the normal ECG in the rabbit given in the referred papers are registered. The pathological changes observed are grouped after simple description. The shape of the ECG just after the detonation as compared with that found some days afterwards is as a rule different, some changes being very short-lasting (Fig. 2), some on the other hand appearing only after some hours (Fig. 3) up to some days. It thus seemed reasonable to describe both separately.

Of the whole material exposed, 127 rabbits (65.5 per cent) showed certainly pathological changes in the ECG of short or long duration. Nineteen out of the 62 rabbits (30.6 per cent) exposed in the open field had pathological ECG while the corre-

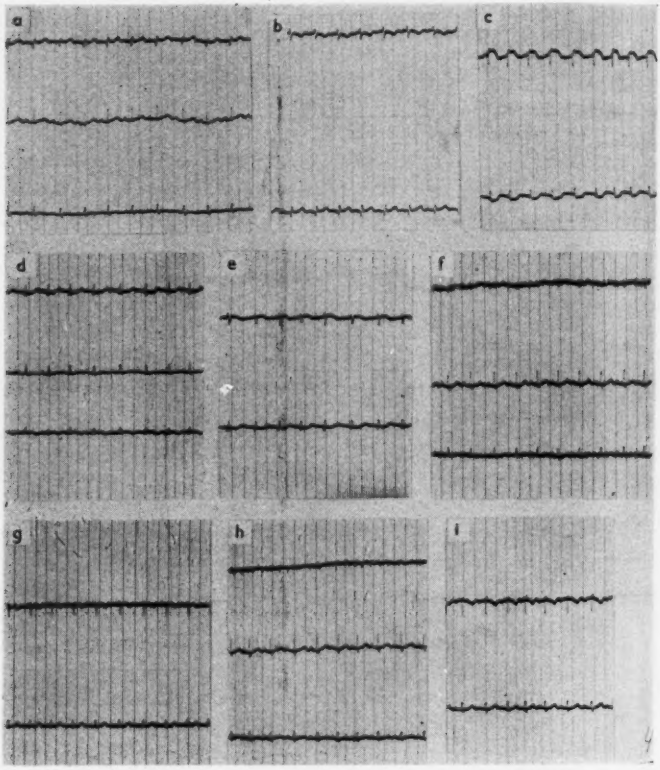

Fig. 2. Rabbit B 14. Exposed in the detonation chamber to a maximum blast pressure of 11.9 kgf/cm² and an impulse of 32.9 gf.sec./cm².

Quotients of lung hemorrhage $\left(\frac{W_1}{W_0}\right)$: left lung: 2.32 and right lung: 3.20.

ECG in standard limb and chest leads

a and b: I, II, III and R, L from above respectively before the detonation.

c: 3 minutes after the detonation. Leads R and L with monophasic deformation of the initial complex.

d and e: standard limb leads and chest leads respectively 6 hours later. Pathological pattern only in the chest leads. The elevation of RS—T in R is less pronounced in e than in c.

f, g and i: the same leads, 3 days and 7 days after the detonation respectively. ECG normalized.

sponding figures in the second, the detonation chamber exposed group, were 108 out of 132 animals (81.8 per cent).

In most animals both an initial bradycardia and a lowering of

Table 1.

ECG changes in 68 rabbits showing initial changes after the detonation.

ECG change	Number of animals with change
Premature beats	32
Injury potential	12
Auricular fibrillation	9
Depression of RS—T and T	9
Ventricular tachycardia	1
Bundle branch block	1
A—V block I	1
Nodal rhythm	1
A—V dissociation	1
Wandering pacemaker	1
Changes in ECG of 9 of these rabbits, initially pathological and later normalized.	
Injury potential	4
Premature beats	4
Auricular fibrillation	1

8 of these died in a couple of minutes afterwards.
9 of these were normalized in a couple of minutes afterwards.
51 of these remained pathological in later controls.
111 rabbits did not show any definite changes initially, but in later controls after the detonation 52 of these were pathological in ECG.

the voltage occurred, irrespective of the future fate. These changes have been excluded from the pathologic reactions.

It is important to know when the ECG changes occur and how long-lasting they are. Sixty-eight of the 127 (53.5 per cent) rabbits, the ECG of which became pathological, had initial ECG changes. Of these 68 animals 8 (12.0 per cent) died immediately, 51 (75.0 per cent) were still pathological later (some minutes to some hours), (Figs. 4, 5, and 6), whereas 9 (13.0 per cent) were normalized later on (Fig. 2).

Some minutes to a couple of hours after the exposure 103 had pathological changes, 52 of which were normal (Fig. 3) and 51 pathologic initially (Figs. 4, 5 and 6).

The question of the duration of ECG changes in blast injury and the possibility that changes might occur not until some days after the exposure necessitated an experimental group, the rabbits of which were controlled several times not only the day of exposure but also each day up to a week after the detonation. This material consists of 15 rabbits. Four of these showed no

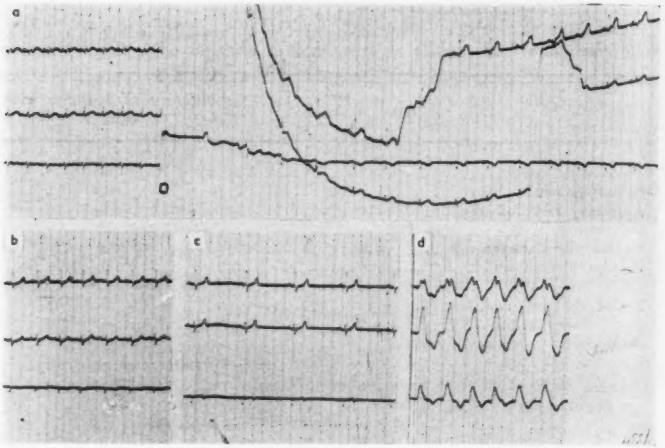

Fig. 3. Rabbit T 12. Exposed in the detonation chamber to a maximum pressure of 14.8 kgf/cm² and an impulse of 27.5 gf.sec./cm².

Quotients of lung hemorrhage $\left(\frac{W_1}{W_0}\right)$: left lung: 2.95, and right lung: 2.74.

ECG in standard limb leads.

a: before, during and immediately after the detonation. 0 marks the detonation. There is a moderate bradycardia.

b: 42 minutes after the detonation. The heart rate higher. RS—T slightly depressed in II.

c: 30 minutes later. Sinus bradycardia.

d: 8 minutes later the rabbit died in ventricular tachycardia.

definite pathological changes in ECG after the blast. Two were normal up to 4 hours after the exposure, but later on certain changes occurred. Of the 13 rabbits which survived more than one day 7 had changes left, 4 of them even one week afterwards. In one rabbit (Fig. 2) changes in ECG were present only during the first day and only in the chest leads. The controls 3 and 7 days after the detonation showed normal patterns in this animal.

Discussion.

The injuries caused by shock waves from a detonating high explosive have been analyzed by among others CLEMEDSON et al. (1949—54). As far as the heart is concerned mainly two mechanisms of significance have been discussed; firstly reflexes evoked in the damaged lungs and secondly the blast pressure

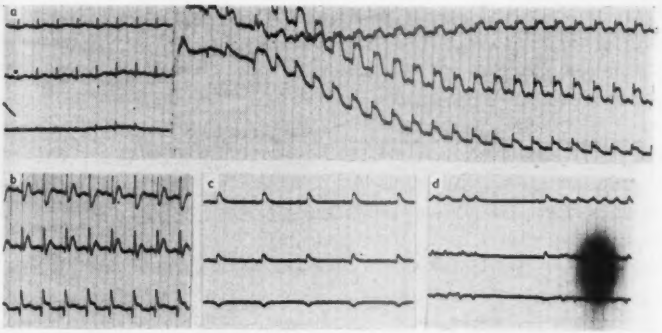

Fig. 4. Rabbit K 17. Exposed in the detonation chamber. Maximum pressure: 14.7 kgf/cm²; impulse: 31 gf.sec./cm². Quotients of lung hemorrhage $\left(\frac{W_1}{W_0}\right)$: left lung: 3.45, right lung: 3.09. ECG in standard limb leads. a: before, during and immediately after the detonation. Monophasic deformation occurs in the very first heart cycles after the detonation. b, c and d: 1, 5, 13 and 14 minutes later show the changes ending in periods of ventricular tachycardia. The animal died spontaneously.

Table 2.

ECG changes in 103 rabbits showing changes only after several minutes to some days after the detonation.

ECG change	Number of animals with change
Depression of RS—T and T	27
Injury potential	24
A—V block III	13
Idioventricular pacemaker	9
Premature beats	8
A—V block II	4
Auricular fibrillation	4
Auricular flutter	3
Nodal rhythm	3
Bundle branch block	3
Ventricular tachycardia	2
Sino-auricular block	1
Wandering pacemaker	1
A—V dissociation	1

70 of these animals died spontaneously.

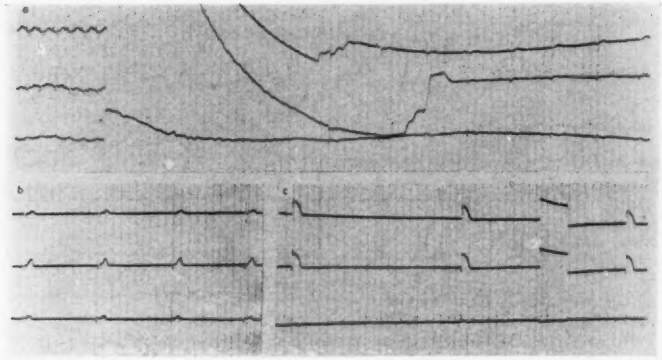

Fig. 5. Rabbit TK 2. Exposed in the detonation chamber. Maximum pressure: 15.2 kgf/cm², impulse: 23.2 gf.sec./cm².

Quotients of lung hemorrhage $\left(\frac{W_1}{W_0}\right)$: left lung: 3.03, right lung: 2.11.

ECG in standard limb leads.

a: before, during and immediately after the detonation. The heart rate changes from more than 300/min. to less than 100/min.
b: 30 minutes after the detonation. More pronounced bradycardia with an ectopic auricular pacemaker.
c: 4 minutes later. Extreme bradycardia from the same pacemaker. Monophasic deformation of the ventricular complex.

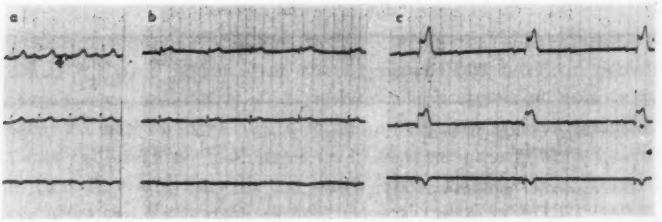

Fig. 6. Rabbit VA 33. Exposed in the detonation chamber. Maximum pressure: 23.0 kgf/cm², impulse 41.6 gf.sec./cm².

Quotients of lung hemorrhage $\left(\frac{W_1}{W_0}\right)$: left lung: 3.27, right lung: 3.20.

ECG in standard limb leads.

a: before the detonation.
b and c: $^1/_2$ and 50 minutes after the detonation respectively. There is an increasing injury potential. In the last record there is a total heart block. The animal died spontaneously.

acting externally on the carotid sinus region. It should however be emphasized, that in addition other types of pathogenetic factors are probable. Circulatory changes to the more or less extensive lung damage, sequelae after hemorrhage, air embolism (BENZINGER 1950, CLEMEDSON and HULTMAN 1954) and direct heart trauma must be considered. O'REILLY and GLOYNE (1941) are of the opinion that the pathology of blast injury is a pulmonary damage caused by violent commotion within the elastic chest. This damage leads to a collapse passing into fatal coma, which begins a few minutes after the explosion without evident external signs of injury. DOWLING and GELFAN (1949) emphasize the anoxic genesis of the damage to the heart.

Some of the effects on the heart of these pathogenetic factors can be demonstrated and followed up with the aid of ECG. Previously some interest has been paid to this topic (KROHN et al. 1942, BARROW and RHOADS 1944, CLEMEDSON 1949, BENZINGER 1950).

Too short a control period before the blast makes it impossible to judge the spontaneous changes in ECG as pointed out by LEVINE (1942). Too short a control period afterwards has given no opportunity to estimate the time of onset and the duration of the changes. Some of the changes caused by the blast injury do not appear in the standard limb leads but only in the chest leads, as observed in the present work. Due consideration has not been paid to these important facts previously.

The present material consists partly of CLEMEDSON's (1949) material, partly of later experiments, in which the above-mentioned views have been regarded.

The most frequently observed manifestations of blast injury in ECG, as observed in the present investigation, are the previously described initial bradycardia and a lowering of the voltage. The former is shown to be due to reflexes from the damaged lungs and partly from the carotid sinus (CLEMEDSON 1949, CLEMEDSON and PETTERSSON 1953), the latter is apparently caused by changes in the conduction tissues surrounding the heart. A low voltage is sometimes observed several days after the blast injury and in these cases also intracardiac causes might be of importance.

The blast injury is initially caused by a very short-lasting positive pressure wave followed by a somewhat more long-lasting low pressure phase. This effect must be extremely instantaneous and should appear in ECG immediately after the detonation. In fact not only the extreme bradycardia and low voltage but also

other pathological changes in ECG are recognized in the very first seconds. Leaving the bradycardia and low voltage out of consideration more than one third of the animals in the present material showed abnormal changes in this period. Among these changes, especially different types of arrhythmia appear in a high percentage. Premature beats, in the majority of cases of ventricular origin and auricular fibrillation are dominating. Twelve of the rabbits got distinct elevation of RS—T very soon (Figs. 2, 4 and 6). Some of the changes are, however, of a transient type (Fig. 2) as illustrated by 13 per cent of those rabbits which initially after the blast had pathological ECG and after some minutes, hours or days were totally normalized and remained so for the whole time of observation. The rest of the rabbits, becoming pathological in ECG immediately showed still abnormal ECG findings of one pattern or another as far as they were followed up.

The absence of initially pathological ECG changes does not exclude a change occurring later on. In the present material 17 rabbits with normal ECG immediately after the detonation showed abnormal ECG some minutes later, whereas 35 remained normal until some hours up to one day after the exposure (Fig. 3). As the older part of our material consists of rabbits with controls only some minutes to some hours afterwards, the figures of the blast injury being reflected in ECG are minimum values. In the group of 15 rabbits thoroughly controlled before and several times up to one week after the explosion 11 rabbits became pathological in ECG but 2 of them not until the day after the exposure.

In the attempts to cathalogize the ECG changes observed only the most dominating pattern of the individual record is included in the tables. It should, however, be pointed out that rather often there are simultaneous combinations of changes in ECG, but as these combinations are several it is impossible to describe all. There are some differences between the firstly occurring ECG changes and those registered some minutes to some days afterwards. Different types of arrhythmia are more dominating initially than later on. This is especially true of the bradycardia but also of the premature beats.

The elevation of the RS—T segment present in about the same proportion in the different stages of observation is in some cases of remarkably short duration. In 7 rabbits it was not only of transient nature but also followed by a normal ECG (Fig. 2).

In the attempts to discuss the genesis of the RS—T elevation the time of onset must be considered. The instantaneously occurring RS—T elevation cannot possibly be secondary to any other injury but must be the result of a direct trauma to the heart, *i. e.* a *commotio cordis*. The elevation of RS—T occurring some minutes later appeared in some cases only in the right chest lead (Fig. 2). This fact and the transient type of some of these changes point to a pattern similar to that found in massive pulmonary embolism. The RS—T elevation in some cases appearing later and of more long-lasting type indicates another type of myocardial damage, more of the nature of infarction.

A—V block II and III do not occur initially but only some minutes to some hours after the detonation. In 13 cases total heart block is the final state. The special form of sinus arrhythmia often seen in total A—V block in man (CARLSTEN 1954) is never seen in the present 13 rabbits with total block. The auricular rhythm is in all these rabbits astonishingly regular (Fig. 6). To interpret this difference from man is as yet not possible. The sinus arrhythmia seen in total blocks in man is found to be due to vegetative influence on the heart and is recorded in in other respects relatively healthy persons, whereas the total A—V block in rabbit in the present investigation is recorded with the animal in a moribund state. Maybe this is the reason for the difference.

Depression of RS—T and T is more common in later controls than soon after the detonation. These changes are very seldom of transient nature and are supposed to be signs of coronary insufficiency.

Finally the spontaneous day-to-day variations in the normal ECG of the rabbit should be mentioned again. In the group, where a series of ECG is taken several days before the exposure to blast, such changes sometimes appear. If they had been found after the blast they might uncritically have been estimated as caused by the detonation. Some of these spontaneous changes are on the border-line between normal and abnormal figures given in LEVINE's (1942) and MASSMAN and OPITZ' (1954) papers.

In conclusion it might be stated, that the blast injury as it is reflected in ECG does not give a definite characteristic pattern, that the changes in the first stages often differ from those occurring later on, that some changes are of a transient type, and that pathological changes in ECG after blast injury might appear not until a day or more after the exposure.

Summary.

1. The ECG changes in blast injury have been studied in 194 rabbits, 62 of which were exposed to a detonating charge of TNT in the open field and the remaining 132 animals to a charge of PETN in a specially constructed detonation chamber.

2. In the total material 65.5 per cent showed pathological ECG after the detonation, in the open field material 30.6 per cent and of the rabbits exposed in the detonation chamber 81.8 per cent. An instantaneous, transient and often pronounced sinus bradycardia and a lowering of the voltage are found in almost every case, but these changes are not included in the pathological changes.

3. Premature beats, depression of RS—T and T, injury potential, different kinds of heart block and auricular fibrillation are rather common findings.

4. Some changes in ECG are not manifested until several minutes to some days after the detonation, and some are only of a transient type. The changes in the first stages after the detonation often differ from those occurring later on.

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Hyaluronic Acid and Hyaluronidase in the Aqueous Humour and the Angle of the Anterior Chamber.

By

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A hyaluronidase-sensitive barrier to the outflow of aqueous humour has been described (BÁRÁNY and SCOTCHBROOK 1954, BÁRÁNY 1953). There can be little doubt that this barrier is located in the filtering meshwork at the angle of the anterior chamber. This meshwork can be regarded as a specialised connective tissue and the component primarily affected by hyaluronidase is very probably hyaluronic acid.

In the earlier papers a distinction was made between a component immediately sensitive to hyaluronidase and a relatively resistant component. It was also shown that the barrier is not stable. A decrease in resistance occurred on perfusion with buffer and could also be induced by slight mechanical trauma.

This paper is concerned with an investigation of the properties of the medium permeating the angle structures and their relation to the resistance to flow developed over several hours. In particular a systematic investigation of the hyaluronic acid-depolymerising action of aqueous humour has been undertaken and the effect of mechanical trauma studied in greater detail.

Methods.

The general procedure was as described previously (BÁRÁNY loc. cit.). Cattle eyes were used throughout and experiments were performed at room temperature unless otherwise stated.

Perfusion fluid.

The buffer had the composition NaCl (0.144 M); sodium barbital (0.0013 M); barbital (0.0069 M); pH 7.2. Before use it was filtered through a sintered glass filter (Jena G 5). This perfusion fluid will be called 'buffer'.

Fresh aqueous for perfusion was removed just before use from cold eyes through a platinum-iridium needle fused into glass and connected with an all-glass syringe by a piece of rubber tubing.

Stored aqueous for perfusion was removed from cold eyes treated in the same manner as the experimental ones. Before puncturing, the cornea was wiped dry with blotting paper but aseptic precautions were not aimed at. The syringe was emptied into a polythene bottle standing in a mixture of solid CO_2 and alcohol. The aqueous was carried to the laboratory in a frozen state and kept at — 20° C till before use when it was thawed in luke-warm water, bubbled with 5 % CO_2 in N_2, centrifuged and the supernatant used immediately. After bubbling its pH was about 7.3.

Perfusion technique.

Perfusion for less than 1 hour was effected in the horizontal burette arrangement described previously with the improvement that platinum-iridium needles with glass sockets and all-glass syringes were used throughout. The perfusion pressure was always 23 cm of H_2O.

Perfusion for longer periods was carried out with a special recording apparatus. The perfusion fluid was kept in polythene bottles through the cap of which a stream of 5 % CO_2 in N_2 could be circulated. Each bottle was suspended on the arm of a photoelectric balance, with the fluid surface about 23 cm above the eyes. The perfusion fluid, which passed from the bottles to the eye through thin polythene tubing came in contact with glass, rubber, platinum-iridium and polythene only. There were 6 photoelectric balances and the weight of the bottles was recorded every 3 min. on a 6 point print recorder.

In some experiments the perfusion fluid leaving the eye was collected. The conjunctiva was dissected away up to the limbus and the eye suspended with the cornea facing downwards. A barrier of blotting-paper was wrapped below the equator to prevent extra-ocular tissue fluid from mixing with the perfusate. The first drops of perfusate, which contained red blood cells were discarded. In most of these experiments no special precautions were taken to prevent evaporation.

In some experiments the accelerated breakdown of the barrier after slight mechanical trauma was studied ('Massage'-effect of BÁRÁNY, 1953). In order to elicit the effect the perfusion was interrupted and 0.5—0.7 ml of fluid drawn back into the burette and reinjected into the eye, the pressure never exceeding the perfusion pressure. This procedure was repeated 5—10 times if not otherwise noted and will be known as 'massage'.

A special arrangement was set up for washing of the anterior chamber without altering its state of distention. Two identical 10 ml all-glass

syringes were mounted together in such a way that the plungers had to move in opposite directions; one syringe filling while the other was emptying. Each syringe was connected by way of a 3-way tap to the anterior chamber of the eye, one of them to a reservoir of washing fluid and the other to a discard. In this way a very thorough washing of the anterior chamber could be performed.

In order to mix the contents of the anterior chamber without inducing a massage-effect magnetic stirring was used. The stirrers were pieces of straight steel wire, 0.5 mm diameter, 10 mm long, with tapered and smooth ends and coated with pure gold. They were introduced into the anterior chamber through a puncture made with a 0.5 mm platinum-iridium needle. The same puncture was then used for the perfusion cannula, which was 0.6 mm in diameter. No leaks ever occurred. The stirrers were not rotated but simply lifted and dropped many times with a magnet.

All rubber tubing was 'RLP Latex Surgical Tubing'. It was washed for many hours with tap water and given a final rinse with distilled water. Pyrex distilled water was used in all critical applications. Taps were greased with pure paraffin jelly.

Preparation of the aqueous humour concentrate.

Aqueous humour was removed from cattle eyes through a stainless steel needle and an all glass syringe. The condition of the eyes is recorded in Table 2; p. 274. In some cases the pooled aqueous was divided into portions of known volume and part injected into the anterior chamber, left for 2—3 hours at room temperature and then removed. In others, after removal of the original aqueous, 0.15 M NaCl or the perfusion-buffer was injected into the anterior chamber, left at room temperature and then removed. After final removal from the eye all fluids were cooled to 0° and dialysed against distilled water at 3° for 24 hours in cellophane sacking (Visking, Chicago). The non-diffusible fraction was centrifuged to remove a small insoluble residue, frozen and dried by sublimation. The solid was dissolved in 0.15 M NaCl, 0.1 M acetate buffer, pH 6, a small amount which was insoluble was discarded.

Hyaluronic acid preparations.

Two preparations of hyaluronic acid were made from human umbilical cords by the method of JEANLOZ and FORCHIELLI (1950). Both preparations corresponded to fraction VII of these authors. One sample was not completely freed from protein by the Sevag technique. Aqueous solutions of these materials were made and passed down a column of Amberlite IR 120 to remove cations. Measured volumes of the effluent were dried at 105° and further volumes analysed for the nitrogen, hexosamine and sulphur content. The composition of the ash-free dry material was

	% N	% Hexosamine	% S
Sample A	4.25	38.1	0.35
Sample B	3.64	44.0	0.13

The concentration of these materials will be expressed in terms of the free acid, not the salt.

A partially purified preparation of the hyaluronic acid-protein complex of OGSTON and STANIER (1950) was made from horse synovial fluid, obtained from the slaughter-house. The synovial fluid was centrifuged and the clear fluid (50 ml) removed from the sediment and a fatty material which collected at the surface. It was filtered through a sintered Jena-glass filter 17 G 5 ('Standardwert' 626 mm Hg, max. pore size according to Bechold 1.21 μ). The residue on the filter was suspended in water and washed (6 $\times$ 100 ml of water). All these operations were carried out at 4°. Finally the ultrafilter residue was suspended in 0.15 M NaCl and brought to pH 8 with Na_2CO_3. After about two days a solution was obtained and the pH had fallen to 7. The solution was clarified by high speed centrifugation at + 2° C and used directly in the viscosity experiments. The concentration of the solution was obtained by dialysing known volumes against N/1,000 HCl and then distilled water and drying the dialysed solution at 105°. The N content of the dry residue was obtained and the hexosamine content of a further sample determined without drying. The composition of the preparations were

	% N	% Hexosamine
Synovial fluid hyaluronic acid 1	9.8	17.2
» » » » 2	7.9	18.5

The concentrations of the solutions will be expressed as mgm combined hexosamine/ml. This material will be called 'Synovial fluid-hyaluronic acid'.

Hyaluronidase preparations.

In the experiments with the aqueous concentrate the testicular hyaluronidase 'Rondase' (Evans, Medical Supplies, London) was used as the standard. In most of the perfusions the testicular hyaluronidase 'Hyalase' (Leo, Hälsingborg, Sweden) was used, in a few, the testicular preparation 'Kinaden' (Schering A. G., Berlin) or the staphylococcal enzyme 'Hyason' (N. V. Organon, Oss, Holland) were used. 100 of the viscosity reducing units used by the manufacturers to specify the activity of Hyalase are equivalent to 600 μg of Rondase, or 80 viscosity reducing units of the manufacturers of Hyason or 4.35 of the Schering units describing Kinaden.

These relative activities were determined viscosimetrically on a sample of hyaluronate from umbilical cord by Messrs. Leo, Hälsingborg. All enzyme activities in this paper will be converted to the equivalent viscosity reducing units (v. r. u.) of Hyalase Leo. This facilitates comparison, but is subject to the reservations made by HUMPHREY and JAQUES (1953) for comparison of enzyme activities assayed on different substrates. Hyalase Leo is the enzyme studied under Code No. III by MALMGREN (1953); Hyason is preparation D of CHAUNCEY and others (1953).

With regard to specificity, Hyalase and Hyason have been compared by reductometric and turbidimetric assay (WOODIN unpublished).

Hyalase hydrolyses hyaluronic acid and chondroitin sulphate, Hyason only the former.

Analysis of the aqueous humour concentrate.

For total nitrogen. The total volume of the concentrate was noted and a small volume dialysed exhaustively against distilled water. The nitrogen content of the dialysed solution was obtained and assuming that it is all combined in proteins with a nitrogen content of 16 % the protein content calculated.

For hyaluronidase activity. The turbidimetric method (PEARCE 1953a) was used to detect hyaluronic acid depolymerase activity in the concentrated aqueous. In each determination tubes were set up containing in 1 ml, respectively, hyaluronic acid; hyaluronic acid and a series of hyaluronidase concentrations; aqueous humour concentrate; and aqueous humour concentrate with added hyaluronic acid. The amount of added hyaluronic acid was 200 μg in all cases. In the later determinations tubes were incorporated containing also aqueous humour concentrate with added hyaluronidase. All solutions contained 0.15 M NaCl, 0.1 M acetate buffer, pH 6. Initially incubation was for 30 min., later this was increased. The curves of PEARCE (1953a) indicate that even with low enzyme concentration there is a slight increase in the degree of depolymerisation after this time. After the incubation 5 ml of serum albumin reagent were added and the optical density measured at 540 mμ in a Unicam spectrophotometer using 1 cm cells.

Nephelometric Determination of the Hyaluronic Acid in Native Aqueous Humour.

The intensity of the scattered light was measured in a nephelometer consisting of a modified photofluorometer (Coleman Electric Co. Maywood, Ill.). A tungsten lamp supplied with stabilised current was fitted in place of the mercury lamp. Light incident on the sample was passed through filter No. 14—218. A sheet of polaroid was inserted in the filter holder between the sample and the photocell and orientated to reduce the intensity of light reflected by the sample tube to a minimum. The sample tube was fixed permanently in place by a metal clamp and solutions were changed by a syringe. Scales were fixed to the dials marked 'Bal' and 'Blk'. The sensitivity controlled by 'Std' was kept at a maximum and the scale on 'Blk' calibrated in terms of the galvanometer scale. By manipulation of 'Bal' and 'Blk' the effective range of the galvanometer became 600 scale divisions.

Solutions of fluorescin were used as standards to determine variations in the sensitivity of the instrument. The stock solution contained 30 mg of sodium fluorescin in 200 ml 75 % methanol and dilutions in 1 % Na_2CO_3 in the range $5 \cdot 10^{-5}$ to $40 \cdot 10^{-5}$ gave fluorescent light of an intensity of the same order of magnitude as the light scattered by aqueous humour when acidified together with serum albumin.

The effect of pH on the development of turbidity by aqueous humour mixed with acidified serum albumin is shown in Fig. 1. The serum

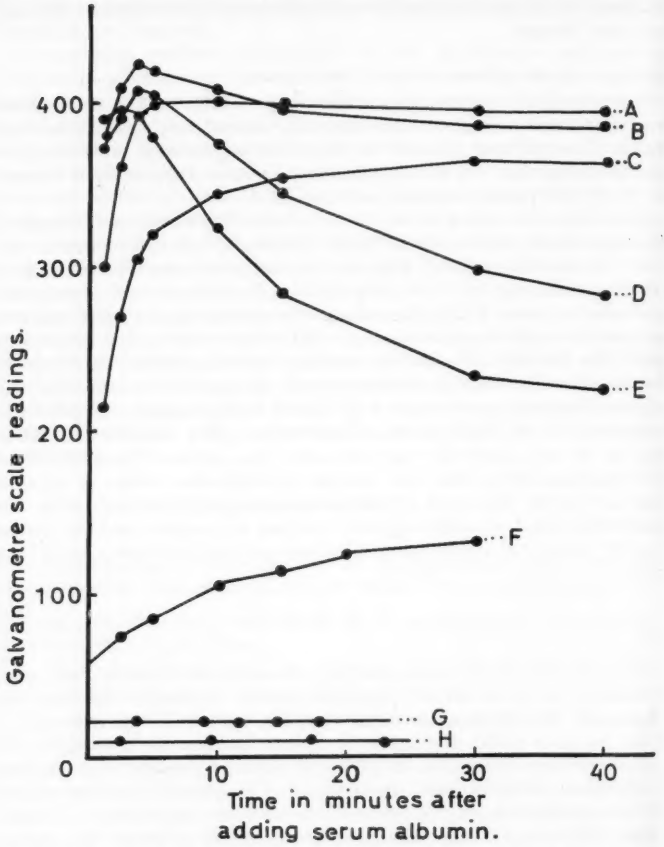

Fig. 1. Effect of pH on the development of turbidity by aqueous humour acidified together with serum albumin.

The final pH of the mixed solutions was: A. 4.10; B. 4.25; C. 3.85; D. 4.35; E. 4.50; F. 3.70; G. 4.15. Curve G. is for aqueous humour after incubation with 10 v. r. u. Hyalase/ml for 30 minutes. Curve H. is for serum albumin 0.1 % in 0.05 N acetic acid.

albumin reagent for these experiments was made from 0.1 % serum albumin in 0.05 N acetic acid. To samples of this, small amounts of 1 M sodium acetate were added to give a series of solutions with a pH range 3.10—4.40. The curves in Fig. 1 are for mixtures consisting of 6.5 ml of serum albumin solution, 1 ml aqueous humour, 0.1 ml methyl gelatin (0.1 % in 0.9 % NaCl), and 0.1 ml distilled water.

From Fig. 1 it is clear that the turbidity is most stable when the pH is 4.15—4.05, and routine measurements were made in this range. For routine measurements 1 ml of aqueous was mixed with 0.1 ml methyl gelatin (0.1 % in 0.9 % NaCl) and 0.1 ml acetate buffer (1.5 M pH 5). The tubes were degassed in vacuo, 6.5 ml of serum albumin, 0.1 % in 0.07 N acetic acid added and the mixtures transferred to the nephelometer. For each solution the development of the turbidity was followed and comparisons made when it was most stable (10—15 minutes after addition of the serum albumin). The pH of the mixture was 4.1, solutions with a pH outside the range 4.15—4.05 were discarded.

Viscosimetry.

Viscosity measurements were made in the capillary microviscosimeters described by CANNON and FENSKE (1938). The viscosimeters had a flow time of 15 seconds for 0.4 ml of water. In the determination of the viscosity reducing activity 0.4 ml of the solution under investigation was placed in a tube and 0.1 ml of the synovial fluid-hyaluronic acid added from a blood pipette. The solutions were mixed and 0.4 ml transferred to a viscosimeter and incubated at 37°. Flow times were measured during 45 minutes. In some cases the solvent was the perfusion buffer, which in this context will be referred to as NaCl, Na-Barbital buffer.

Results.

The Massage Effect.

This name has been given to the resistance drop that is often but not always induced by manipulation of the contents of the anterior chamber. (BÁRÁNY 1953.) The effect was first observed frequently following attempts to add foreign material to the anterior chamber by repeated aspirations and refillings (Fig. 6, BÁRÁNY 1953). Later it was found that even partial withdrawal and reinjection of the anterior chamber contents themselves could elicit the effect. It was thought that fluctuations in the distention of the eye ball were the cause, and special experiments were designed to permit strong stirring or washing out of the contents of the anterior chamber at constant distension. Even under these conditions, and with fresh aqueous removed from cold eyes as the washing fluid, a rapid drop in resistance would sometimes occur, Fig. 2.

We are as yet not able to define the conditions under which a massage effect is certain to appear, nor are we able to specify a perfusion procedure where it can be avoided with certainty.

It seems to be associated with slight mechanical trauma to the walls of the anterior chamber and it is possible that it cannot be avoided altogether even in the simplest experiment, that of setting up an eye for perfusion. Thus it could be responsible for

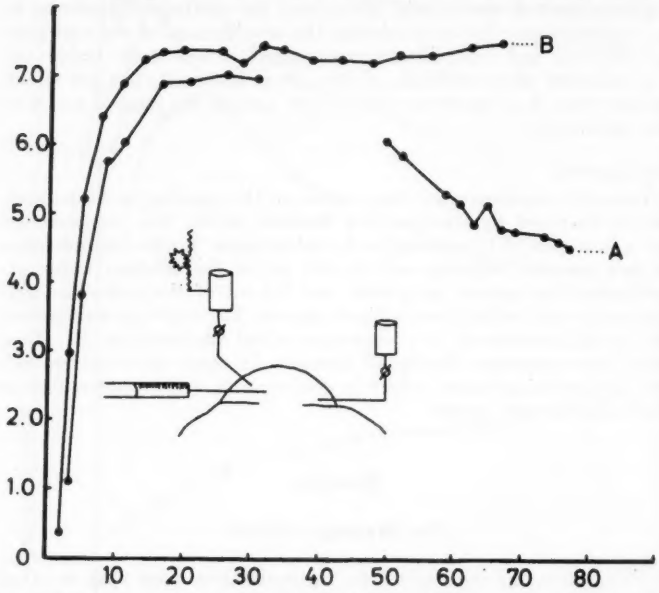

Fig. 2. Effect of washing out the anterior chamber with aqueous humour without change in pressure.

Abscissa: time in minutes. Ordinate: resistance, expressed as time in minutes needed for 0.1 ml to enter the anterior chamber.

A: experimental eye, B: control eye from same animal.

During the break in curve A, the anterior chamber was washed through with 10 ml of fresh aqueous by means of 2 extra needles leading from 2 reservoirs. The perfusing burette was left connected with the chamber during the whole procedure and the height of the one reservoir continuously adjusted so as to keep the fluid in the burette at constant level. The aqueous in the reservoirs was under paraffin.

part of the 'spontaneous' decrease in resistance which is usually seen on prolonged perfusion.

The resistance drop caused by massage as a rule takes the form of a smooth decrease, as in Fig. 2. Sometimes, however, when perfusion is interrupted by strong massage, a considerable drop in resistance is found to have occurred before the start of the second period of perfusion.

We do not know whether this immediately-appearing loss in resistance has the same cause as the more gradual resistance drop. Fig. 3 is a case where one process would seem to be sufficient to explain the findings while Fig. 4 illustrates a case where

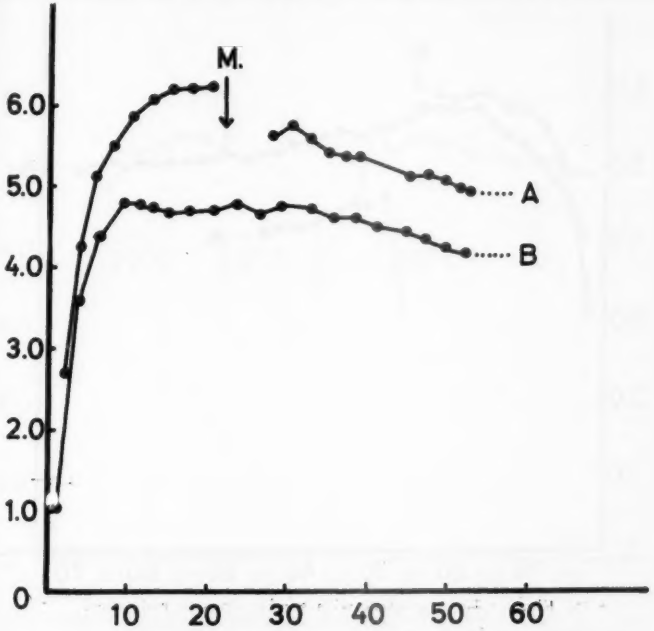

Fig. 3. Massage-effect of the usual kind.

Coordinates: see Fig. 2.
A: experimental, B: control eyes. M: massage.

two processes would seem to be at work. This latter type of curve is only rarely encountered and only after rather violent massage.

After the drop induced by massage the resistance of the chamber angle is less affected by hyaluronidase. Massage, after hyaluronidase has no effect and it is possible therefore that it affects the hyaluronidase-sensitive part of the barrier.

Temperature dependence of the massage effect.

In preliminary experiments it was established that cooling and rewarming the eye during perfusion did not cause a breakdown in the resistance. Subsequently, the temperature dependence

of the massage effect was studied with the usual burette arrangement and with the eyes immersed in 0.9 % NaCl at various temperatures.

If an eye is perfused at room temperature and a massage effect induced, the progress of the effect can be slowed down,

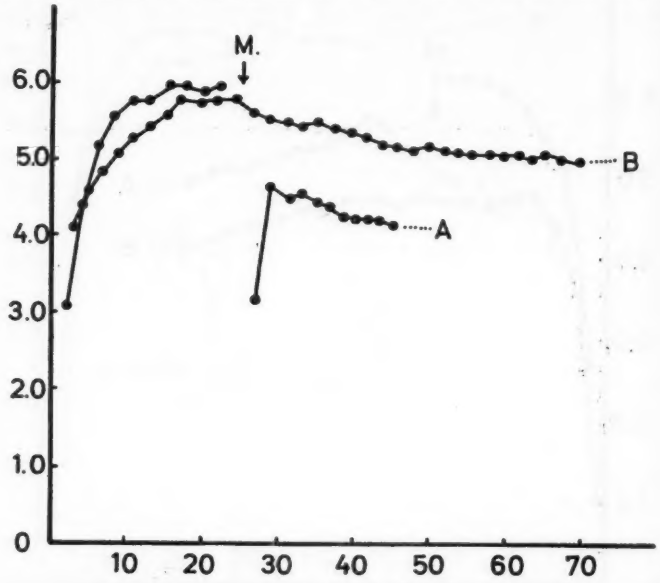

Fig. 4. Massage-effect with sudden drop in resistance.

Coordinates: see fig. 2.
A: experimental, B: control eyes. M: massage.

or temporarily stopped, by immersion of the eye in ice-cold saline. Usually the breakdown of the barrier proceeds on rewarming to room temperature. Fig. 5 illustrates an experiment of this sort.

If massage is performed while the eye is kept in ice-cold saline, a resistance drop is sometimes found to have taken place when measurements are resumed at room temperature. In other cases, however, the original resistance is reached on rewarming and is followed by a gradual decay. The latter type of result is consistent with there being a primary effect of massage which takes place in the cold and a secondary effect, the gradual breakdown, which is more or less inhibited at low temperatures.

In as much as the effect of massage was sometimes delayed by cold, the possibility was investigated of changing the contents of the anterior chamber by washing in the cold room. In particular it was considered that if the massage effect is due to the action of a normal constituent of the aqueous humour, then its removal

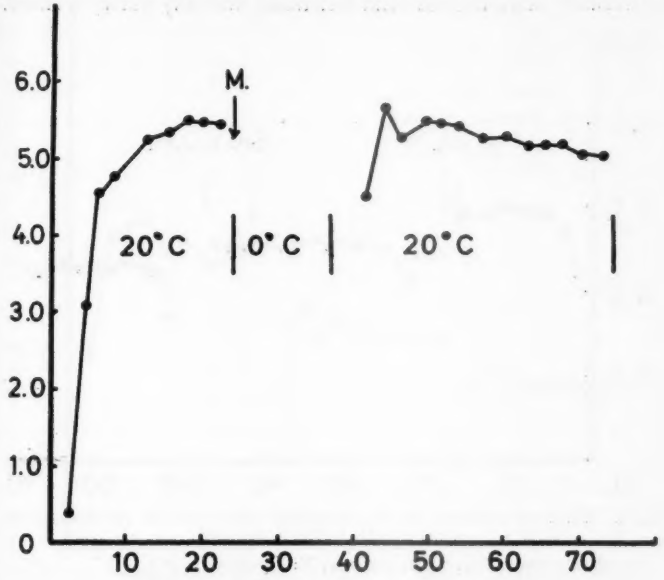

Fig. 5. Temporary inhibition of massage-effect by cooling. Coordinates as in Fig. 2. M: massage. Perfusion fluid aqueous.

by cold washing would prevent the appearance of the effect but preserve the barrier intact.

Two series of experiments were made in the cold room at 4° C. In one series, eyes were connected with a large reservoir of cold buffer through one needle in the anterior chamber, a second needle providing free exit for the washing fluid. Several hundred ml of cold buffer were allowed to flow through the chamber in the course of several hours. After this washing, the needles were pushed through the opposite side of the cornea, the eyes put into saline at room temperature for 15 minutes, and ordinary perfusion performed with buffer. In the second series, the double syringe arrangement mentioned under "Methods" was used for

the washing. The procedure took much less time and the turbulence induced was much stronger in this series. The amount of buffer put through the chamber was 60, 90 or 120 ml, in this range the amount seemed to make no difference.

The expectation that cold washing could be carried out without appreciably affecting the total resistance was only partly realized.

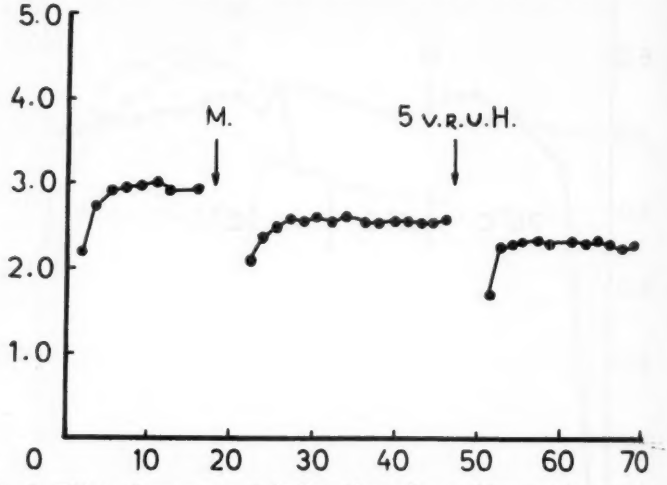

Fig. 6. Effect of massage and hyaluronidase after washing out the anterior chamber with buffer in the cold.

Coordinates as in Fig. 2. M: massage, H: Hyalase.

About half of the eyes treated in this way had a very low resistance. In some of these, Hyalase was given and had little or no effect. In the remaining 12, massage was applied and its effect observed and finally 5 v. r. u. Hyalase injected. Fig. 6 shows a typical result: the resistance was lower after massage, but there was no continuous drop as in ordinary eyes. This behaviour was seen in 7 of 12 experiments; in the remaining 5, a small continuous drop occurred, as in Fig. 7.

Attempts to transfer the massage effect.

If the gradual breakdown after massage were due to a substance liberated by the procedure, the aqueous of a massaged eye could conceivably break down the barrier of a non-massaged one.

In experiments with 8 pairs of eyes, one eye was perfused with pooled aqueous and massaged. Ten minutes later when the

resistance drop had started, the contents of the chamber were magnetically stirred and then drawn back into the perfusing burette. The massaged eye was removed and the second eye of the pair connected with the burette. When about 1 ml of the

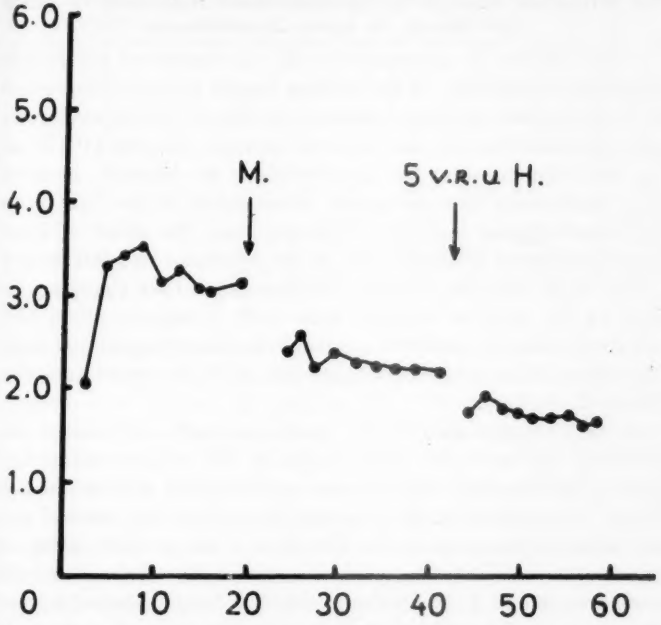

Fig. 7. Effect of massage and hyaluronidase after washing out the anterior chamber with buffer in the cold.

Coordinates as in fig. 2. M: massage, H: Hyalase.

"massaged aqueous" had passed into the second eye, it was magnetically stirred. In none of these experiments did the result of perfusion with "massaged aqueous" differ from the normal course.

In another series of 8 experiments, perfusate from the limbal region of cattle eyes was collected and injected into rabbit eyes. There was no difference between perfusate before and after massage.

Miscellaneous observations concerning the massage effect.

A normal massage effect could be observed in two eyes containing 0.12 mg/ml of diethyldithiocarbamate in the anterior chamber.

Strong massage did not cause a breakdown of the barrier in 3 pig eyes, while a normal massage effect was observed in the eyes of calves.

The Minimum Amount of Hyaluronidase Necessary to Affect the Barrier in Acute Experiments.

It was clearly of importance in the elucidation of the factors governing the stability of the outflow barrier to have information on the hyaluronic acid depolymerase activity of, and the hyaluronic acid concentration in, the aqueous humour. MEYER (1947) has reported the presence of hyaluronidase in aqueous humour. As a preliminary to a systematic investigation of the hyaluronic acid depolymerase activity of the aqueous, the effect of small concentrations of hyaluronidase on the resistance was determined.

The main difficulty in such experiments is that changing the fluid in the anterior chamber may elicit a massage effect and, moreover, that the barrier is not stable to uninterrupted perfusion. The effect of the enzyme has therefore to be discerned against a falling background.

In preliminary experiments, graded amounts of Hyalase dissolved in 0.2 ml buffer were placed in the rubber tubing connecting burette and needles, close to the latter and before perfusion had started. In this manner the enzyme was washed into the anterior chamber by the first 0.5—1 ml of fluid filling up the eye at the start. The perfusion was then continued and the resistance recorded. It was found that 0.125 v. r. u. caused a faster drop than was usually found in untreated eyes, but that the effect of 0.063 v. r. u. was too small to be easily discernible.

In the main series, conditions were better controlled. Two horizontal burette arrangements, Nos. 1 and 2, were set up so that the needle of No. 2 entered the rubber tubing of No. 1 close to the socket of its needle. (See diagram Fig. 8.) A magnetic stirrer was introduced into the eye and perfusion started with No. 1 burette containing pure aqueous. When the resistance had reached its quasi-stable maximum, 0.15 v. r. u. Hyalase in 0.075 ml buffer was introduced by an Agla micrometer syringe into the tubing of No. 1, distally to the point of the needle of No. 2 without interrupting perfusion. At the same time perfusion was shifted to burette No. 2, which contained 0.05 v. r. u. Hyalase/ml aqueous, a concentration equal to that expected in the anterior chamber of the perfused eye once the initial dose had entered.

Table 1.

Influence of 0.05 viscosity reducing units of Hyalase per milliliter on the rate of decay of the barrier.

Resistance drop during 20 minutes in % of initial resistance

Enzyme treated	Controls	Comment
35	1	
38	12	
49	21	
26	4	
11	11	
mean = 32	mean = 10	
29	29	In these experiments, perfusion had to be stopped for refilling the burette during the control runs. This is apt to elicit a massage effect.
31	45	
34	13	
42	34	

Magnetic stirring was employed to distribute the small volume of enzyme given initially. Perfusion with the second burette was continued for at least 20 minutes. The second eye of each pair was used as control and given the enzyme solvent in exactly the same manner. The result of these experiments (Table 1) indicate that a hyaluronidase activity equivalent to about 0.05 v. r. u./ml aqueous is necessary to produce a resistance change comparable in rate to that brought about by massage, (see below, Tables 5 and 6) 32 % drop in 20 minutes as against 10 % in the controls. One of the experiments is shown in Fig. 8.

Action of bacterial hyaluronidase.

Some orienting experiments were made to establish the power of Hyason to cause an immediate resistance drop. It was found that 0.25 of the manufacturer's viscosity reducing units, injected in 0.2 ml of buffer, caused the resistance to drop with about the same speed as 2 v. r. u. of Hyalase. The extent of the drop and the form of the curve were the same with Hyason as with Hyalase.

Hyaluronic Acid and its Stability in the Aqueous Humour.

The non-diffusible depolymerase activity of concentrated aqueous humour.

The depolymerase activity of the concentrate was determined by comparing the turbidities developed, after incubation, by

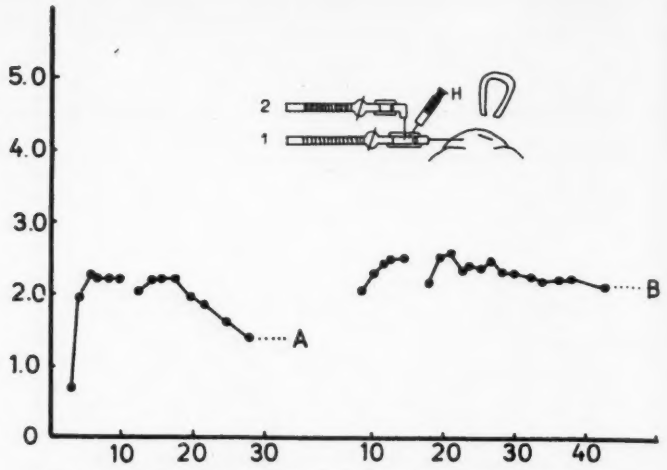

Fig. 8. Effect of low concentration of hyaluronidase on resistance.

Coordinates as in Fig. 2. A: experimental, B: control eye.
During the break in curve A 0.075 ml containing 0.15 v. r. u. Hyalase were injected and perfusion shifted from burette 1, containing aqueous to burette 2, containing 0.05 v. r. u. Hyalase/ml aqueous.

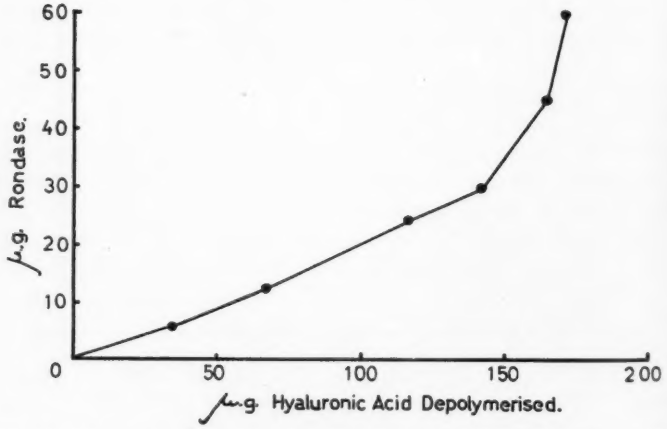

Fig. 9. Effect of concentration of Rondase on hydrolysis of hyaluronic acid, estimated turbidimetrically. Incubation time 30 min.

hyaluronic acid and by aqueous concentrate separately with that of the mixed solutions. The action of various concentrations of Rondase on hyaluronic acid is apparent from Fig. 9. From this curve and the relation between the turbidity developed by, and the concentration of, the hyaluronic acid (Fig. 10) it was concluded that an activity equivalent to that of 3 μg of Rondase

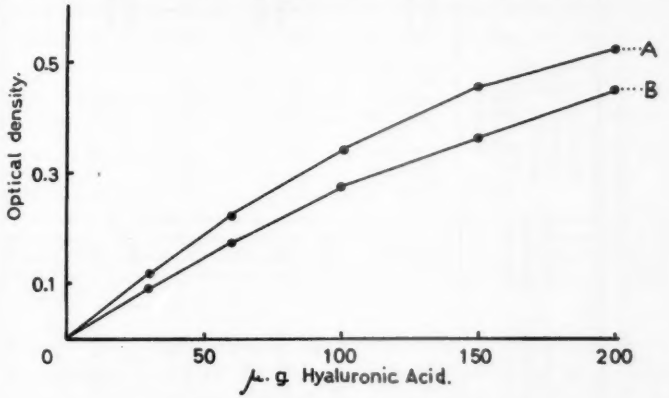

Fig. 10. The turbidity developed by hyaluronic acid from umbilical cords. 1 ml of a solution containing hyaluronic acid in 0.1 M acetate buffer, 0.15 M NaCl, pH 6 was treated with 5 ml serum albumin, 0.1 % in 0.1 M acetate buffer, brought to pH 3.75 with HCl. A and B are for different preparations.

could be detected by this method. This value, together with the concentration factors enabled an upper limit to be set to the hyaluronidase activity of the original aqueous humour. The results, summarised in Table 2 support the conclusion that the aqueous humour, or extracts of the anterior chamber, contain less depolymerase than the equivalent of 0.05 v. r. u. of Hyalase Leo or 0.3 μg of Rondase.

Concentrated aqueous humour consistently gave a turbid solution when acidified with serum albumin. 0.5 ml of a 10-fold concentrate gave a turbidity equivalent to that produced by 25—75 μg of our hyaluronic acids from umbilical cords. Accordingly in the later experiments mixtures of the concentrate and Rondase were incorporated into the assay. The hyaluronidase consistently decreased the turbidity producing material of the aqueous.

Inactivation of a hypothetical hyaluronidase of the aqueous

Table 2.

Hyaluronidase activity of concentrated cattle aqueous humour.

Expt. no.	Treatment before dialysis	Volume of aqueous	% protein in original aqueous	Concentration factor for tests	Equivalence of hyaluronidase activity in Hyalase units/ml original aqueous	Method
2	Aqueous removed from eyes kept 2 hrs. at room temperature	35 ml	0.027 %	17	0.2 v. r. u./ml	turbidimetric 30 min. incubation
3	Aqueous removed 5 min. after death of animal	63 ml	0.015 %	10	< 0.05 v. r. u./ml	»
4a	Aqueous removed from eyes kept 2 hrs. at room temperature	54 ml	0.04 %	10	»	»
4b	NaCl-Na barbital buffer injected and left in anterior chamber 2 hrs. at room temperature. Eyes from 4a	50 ml	0.02 %	10	»	»
5a	Aqueous removed from eyes kept 2 hrs. at room temperature	90 ml	0.014 %	15	»	turbidimetric 90 min. incubation
5b	Pooled aqueous injected (1.5 ml into each of 29 eyes) left 2—3 hrs. at room temperature. Eyes and aqueous from 5a	40 ml	0.03 %	7	»	»
5c	0.15 M NaCl injected into anterior chamber (1.5 ml into each of 32 eyes) left 2—3 hrs. at room temperature. Eyes from 5a	40 ml	0.01 %	7	»	»
6	Aqueous removed from eyes kept 2 hrs. at room temperature	100 ml	0.022 %	10	»	turbidimetric 165 min. incubation
7a	Aqueous removed from eyes kept 2 hrs. at room temperature	100 ml	0.012 %	10	»	»
7b	Pooled aqueous injected into anterior chamber. Left 2—3 hrs. before removal. Eyes and aqueous from 7a	45 ml	0.024 %	5	»	»

Table 3.

Expt. no.	Concentration factor	μg hyaluronic acid in 0.5 ml aqueous concentrate	μg Rondase added	Incubation time	μg added hyaluronic acid depolymerised in absence of aqueous	μg hyaluronic acid depolymerised in presence of aqueous concentrate
3	3	10	15	30 min.	95	95
5a	5	40	12	90 min.	160	170
5b	2.3	20	12	90 min.	160	175
5c	2.3	15	12	90 min.	160	160
6a	10	20	12	60 min.	145	160
6b	10	20	12	60 min.	145	155
6c	5	15	12	60 min.	145	150

Action of hyaluronidase on hyaluronic acid in the presence of aqueous humour concentrate. The reference numbers are those for the experiments recorded in Table 2. The turbidity was determined on 1 ml samples, consisting of 0.5 ml aqueous concentrate, 0.4 ml hyaluronic acid solution containing 200 μg, 0.1 ml enzyme or control.

during the concentration procedure had to be considered. Accordingly 2 mg of Rondase were added to 15 ml of aqueous humour, the solution diluted to 40 ml, and put through the same procedure as the other samples of aqueous: 95 % of the activity was recovered in the concentrate. To test for a hyaluronidase inhibitor in the aqueous concentrate small amounts of Rondase were added to the aqueous concentrate with added hyaluronic acid and the decrease in turbidity compared with that observed in mixtures of Rondase and hyaluronic acid alone. The results, Table 3, indicate that there was no loss in activity of the Rondase under these conditions.

Actually there was an increased breakdown which we ascribe to the additional substrate furnished by the concentrate.

From these experiments it is concluded that the hyaluronidase activity of aqueous is less than the equivalent of 0.05 v. r. u. /ml Hyalase Leo. The presence in the aqueous of hyaluronic acid capable of being a substrate for added enzyme supports the conclusion that there is not a high enzyme concentration simultaneously present. No evidence for the presence of an inhibitor was found.

However, the presence in the aqueous of the substrate does not exclude the simultaneous presence of the enzyme because of the complexity of the kinetics of the hyaluronidase-hyaluronic

acid system. The hyaluronic acid in normal aqueous is very dilute and its reaction with low concentrations of enzyme slow. At higher concentrations the rate would increase but it is becoming apparent that the reaction of hyaluronidase with hyaluronic acid is 'self limiting'. Thus the curves of PEARCE (1953a), confirmed by us, indicate that the rate of loss of the turbidity producing power of hyaluronic acid incubated with hyaluronidase decreases with time. A similar effect has been shown for the liberation of reducing material by RAPPORT et al. (1950) and for the loss of viscosity by JENSEN (1954). The latter author showed that addition of more enzyme to the digest when the rate of reduction of viscosity had become zero produced a further reduction. It is known that hyaluronidase is stable to incubation alone at 37° (PEARCE 1953a). The inactivation of hyaluronidase when incubated with hyaluronic acid thus cannot be ascribed to spontaneous denaturation of the enzyme. The decrease in rate of decomposition of hyaluronate could be due to accumulation of the products of the reaction but attention can be drawn to the suggestion of SPENSLEY and ROGERS (1954) that macroanions can unspecifically inactivate hyaluronidase and other enzymes. It is accordingly possible that hyaluronic acid, simultaneously to being a substrate for hyaluronidase, inactivates the enzyme by virtue of its unspecific macroanion effect. These considerations become of great importance in the analysis of systems containing hyaluronidase and hyaluronic acid in low concentrations. Thus it could be possible for there to be in the native aqueous humour both hyaluronic acid and active hyaluronidase, while on concentration of the aqueous the enzyme may not be detectable because of its inactivation in presence of a higher concentration of substrate.

For this reason we have developed a method to determine the stability of the hyaluronic acid of the native aqueous humour.

The hyaluronic acid of native aqueous humour.

Preliminary measurements indicated that there is present in native aqueous humour a material which, mixed with acidified serum albumin, gives a turbid solution. This turbidity development is prevented by incubation of the aqueous with Hyalase (10 v. r. u./ml 30 min.) before adding the serum albumin (see Fig. 1, p. 262). We have therefore used light scattering after addition of acidified serum albumin as a measure of the hyaluronic

acid in the aqueous. The turbidity produced by hyaluronic acid acidified with serum albumin is dependent upon the concentration of the hyaluronic acid and its degree of polymerisation (PEARCE 1953b). It is not possible therefore to define the concentration of hyaluronic acid in the aqueous from measurements of this sort. For comparison, it can be stated that the scattering produced at 5 μg/ml by hyaluronic acid from umbilical cords was similar to that of pooled aqueous. There is, however, considerable variation in the hyaluronic acid content of different aqueous humours.

We have determined the stability of the hyaluronic acid in isolated aqueous on incubation, its sensitivity to hyaluronidase, and we have investigated the action of "massage".

The action of small concentrations of Hyalase on the hyaluronic acid of aqueous humour is shown in Table 4. Under these con-

Table 4.

Action of Hyaluronidase on the Hyaluronic Acid of the Aqueous Humour.

Nephelometer readings for substrate	Incubation time minutes	Enzyme conc. v. r. u. Hyalase/ml	Decrease in nephelometer reading on incubation
190	300	0.014	20
	300	0.028	45
300	30	0.016	7
	30	0.032	11
	90	0.016	6
	90	0.032	11
	270	0.016	18
	270	0.032	52
185	175	0.022	50
	175	0.015	10
	490	0.022	62
	490	0.015	22

Turbidities refer to 1 ml of incubation mixture, which was pooled aqueous humour, 10 volumes; Hyalase and 0.1 % methyl gelatin in 0.9 % NaCl, 1 vol. The control contained aqueous humour and methyl gelatin in NaCl mixed in the same proportions. The decrease in turbidity refers to the difference between the incubated digest and the incubated control. The turbidity of the substrate is that of the control before incubation. Incubation was at 37°, in stoppered tubes containing an atmosphere of 5 % CO_2 in N_2.

ditions incubation of the aqueous alone does not reduce the turbidity producing power, indeed it is slightly increased on incubation (Tables 5—6). However, on addition of Hyalase to give a final

Table 5.

	No. of eyes	Average resistance drop in the 20 min. after massage	Average nephelometer reading before incubation	Average nephelometer reading after incubation
Control	7	13 %	345	350
Massage.....	7	25 %	340	380
Control	4	14 %	215	220
Massage.....	4	30 %	226	232

Turbidities refer to 1 ml samples of individual aqueous humour samples before and after incubation for 5 hours at 37°. Of the individual samples 2 controls and one aqueous from a massaged eye showed a decrease in the turbidity during incubation. Perfusion was with pooled fresh aqueous, the same batch for all experiments in each group. Incubation was at 37° in an atmosphere of 5 % CO_2 in N_2 for 5 hours.

Table 6.

	No. of eyes	Average resistance drop in 20 minutes	Nephelometer readings		
			before incubation	after incubation alone	after incubation with 0.02 v. r. u./ml Hyalase
Control	8	not perfused	not measured	380	340
Massage.....	4	24 %	352	360	330
Massage.....	4	6 %	365	370	330
Control	4	8 %	264	260	
Massage.....	4	19 %	300	310	
Control	4	Aqueous removed from cold eyes without perfusion	190	195	155
Massage.....	4	Aqueous removed from eyes at room temperature 10 min. after massage, not perfused	215	230	

Readings refer to 1 ml pooled aqueous. Incubation for 5 hrs. at 37° in an atmosphere of 5 % CO_2 in N_2. Perfusion with pooled fresh aqueous.

concentration of 0.02 v. r. u./ml a significant reduction in turbidity is obtained compared with that of aqueous incubated without added enzyme.

Tables 5 and 6 summarize the effect of incubation on the turbidity producing material of aqueous taken from eyes treated in various ways. In these experiments, with pairs of eyes from the same animal, one eye was massaged and the other served as a

control. Some pairs were perfused simultaneously with pooled aqueous and after a time which allowed 20 minutes to elapse after massage, the contents of the anterior chamber were removed. In other pairs, the resistance changes were not recorded.

In the experiments of which the results are given in Table 5, individual aqueous humour samples were analysed, in those of which the results are given in Table 6, the aqueous humours from eyes which had been massaged and the corresponding controls were respectively pooled. In one series a distinction was made between those with a large and those with a small resistance drop. The turbidities produced by the aqueous before and after incubation were noted.

It is clear from Tables 4, 5 and 6 that the hyaluronic acid depolymerising power of aqueous is considerably less than the equivalent of 0.02 v. r. u./ml Hyalase, and that this is true even of the aqueous withdrawn from eyes which had shown a large massage effect.

The action of aqueous humour on the viscosity of hyaluronic acid from synovial fluid.

The smallest amount of hyaluronidase which could be detected by the two previous techniques is only slightly less than that concentration, the presence of which in the anterior chamber induces a change in the resistance similar to that produced by massage. By using the hyaluronic acid-protein complex from synovial fluid we have been able to increase considerably the sensitivity of the assay.

Fig. 11 shows the action of Hyalase on a preparation of this material. A final concentration of 0.004 v. r. u. Hyalase/ml produces a considerable reduction in the viscosity. The sensitivity of the method could no doubt be improved by using lower shear rates, but we have not found it necessary to pursue this possibility.

Aqueous produces a decrease in the viscosity of synovial fluid-hyaluronic acid. As is seen in Fig. 12 (D), the time course of the reduction in viscosity differs from that induced by Hyalase. However after 30 minutes incubation the effect is similar in magnitude to that produced by a final concentration in the digest of 0.005—0.01 v. r. u. Hyalase/ml. Samples of aqueous humour removed directly from 4 cold eyes have differed from each other by less than 5 % in their viscosity reducing action, but samples of pooled, stored aqueous, thawed and bubbled with 5 % CO_2

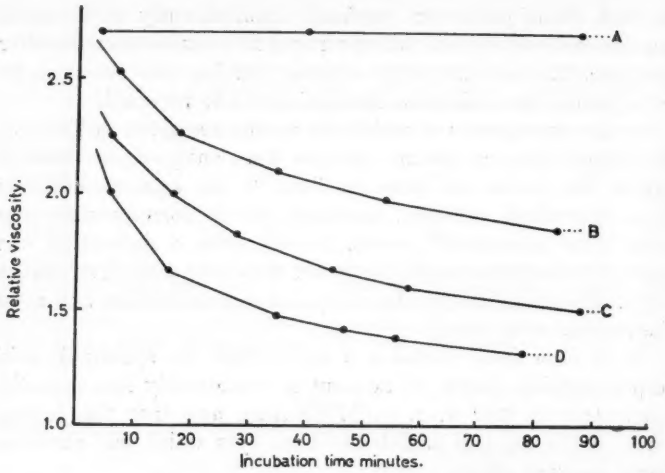

Fig. 11. Action of Hyalase on the viscosity of synovial fluid hyaluronic acid, sample 1.

A Hyaluronic acid in NaCl, Na-barbital buffer (control).
B Hyaluronic acid in Hyalase solution, 0.004 v. r. u./ml digest.
C Hyaluronic acid in Hyalase solution, 0.008 v. r. u./ml digest.
D Hyaluronic acid in Hyalase solution, 0.016 v. r. u./ml digest.
The final concentration of hyaluronic acid glucosamine was 98 μg/ml. Dilutions of Hyalase were made in the NaCl-Na-barbital buffer containing 0.1 % methyl gelatin. Incubation was at 37°.

in N_2, have differed from each other and in general show a lower activity than fresh aqueous.

This viscosity reducing action of the aqueous is more thermostable than that of Hyalase (Fig. 12), it is diffusable and completely inhibited by the addition of 250 μg/ml sodium diethyldithiocarbamate (Fig. 13). Dilution of the aqueous with an equal volume of the NaCl, Na-barbital buffer does not change the activity. These properties are very similar to those of the substances which SKANSE and SUNDBLAD (1943) consider to degrade hyaluronic acid by oxidation. Several such substances are present in aqueous humour. For comparison Fig. 13 shows the action of M/1,100 ascorbic acid on the viscosity of synovial fluid-hyaluronic acid.

It was possible that massage induced the liberation of a depolymerising material from the filtering angle and to investigate this 6 eyes were set up for perfusion and the fluid collected as it

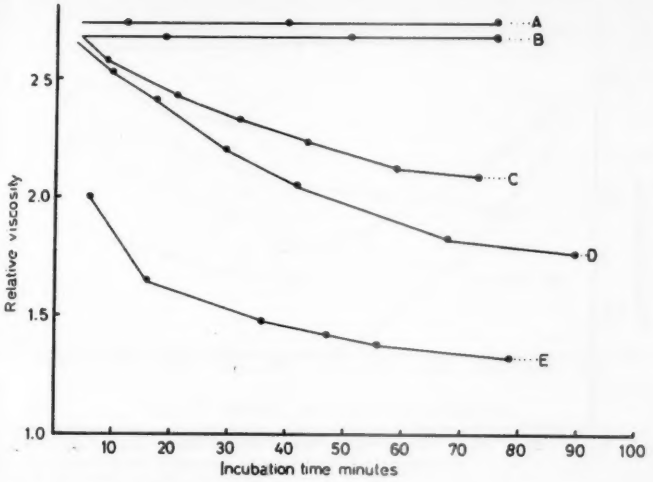

Fig. 12. Effect of heating on the viscosity-reducing activity of aqueous humour.

A Hyaluronic acid in NaCl-Na-barbital buffer.
B Hyaluronic acid in heated Hyalase solution.
C Hyaluronic acid in heated aqueous humour.
D Hyaluronic acid in aqueous humour.
E Hyaluronic acid in Hyalase solution (0.016 v. r. u/ml).
The final concentration of hyaluronic acid glucosamine was 98 μg/ml. The Hyalase solution was made in NaCl-Na-barbital buffer containing 0.1 % methyl gelatin. Heating before incubation was at 70° $\pm$ 5° C for 45 minutes in stoppered tubes containing, in the case of the aqueous humour, an atmosphere of 5 % CO_2 in N_2. Incubation was at 37°. The aqueous humour was collected from cold eyes. Substrate: synovial fluid-hyaluronic acid, sample 1.

emerged from the limbus before and after massage. The viscosity reducing activity of the perfusate was different from that of the perfusing fluid and in general was smaller, but in all cases there was no significant increase in the activity of the perfusate collected before and after massage.

Perfusion for Long Periods.

To facilitate comparison of percentual rates of change the data of this section are presented as geometric means on a semi-logarithmic scale.

Perfusion of eyes before hyaluronidase injection was carried out for long periods with aqueous or with buffer. Fig. 14 shows the resistance of several groups of eyes treated in this way and

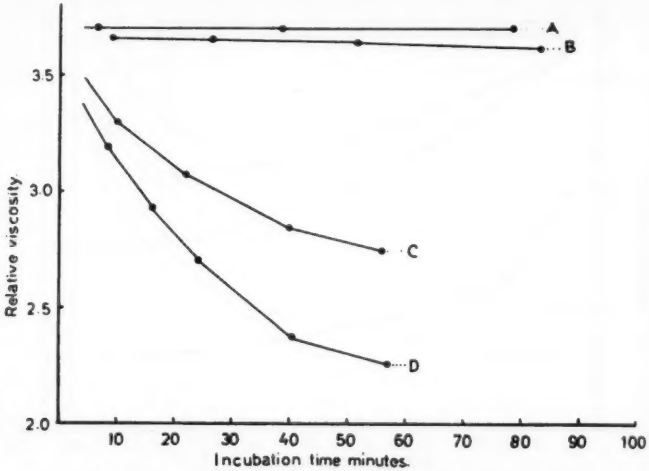

Fig. 13. The action of aqueous humour and of ascorbic acid on synovial fluid-hyaluronic acid, sample 2.

A. Hyaluronic acid in NaCl, Na-barbital buffer.
B. Hyaluronic acid in aqueous humour and 250 μg/ml diethyldithiocarbamate.
C. Hyaluronic acid in M/1,100 ascorbic acid in 0.9 % NaCl.
D. Hyaluronic acid in aqueous humour.
The final concentration of hyaluronic acid glucosamine was 180 μg/ml. Incubation was at 37°.

it is clear that aqueous humour and buffer have different effects. Thus with aqueous as perfusion fluid the resistance changes very little during an initial period and then decreases, while with buffer the resistance drop is rapid from the start. At all corresponding times buffer produces a greater rate of change of resistance than does aqueous. The resistance changes of 6 further eyes perfused with buffer did not differ significantly from those recorded in curve B 1, Fig. 14, but have not been utilised because the experiments on their companion eyes using aqueous were unsuccessful for technical reasons.

Fig. 15 compares the effect of perfusion with aqueous or buffer on the resistance of single pairs of eyes from the same animal.

Injection of Hyalase after 200—280 minutes perfusion with aqueous reduced the resistance by an average of 43 % of its value before the injection, approximately the same percentage drop as generally found after 20 minutes perfusion.

In a further series of experiments 18 eyes were perfused with

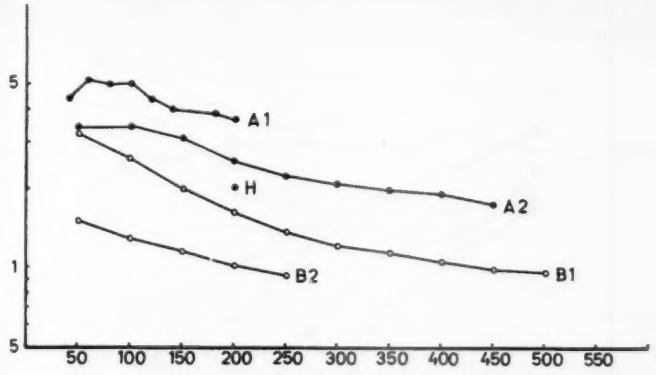

Fig. 14. Perfusion with aqueous or buffer.

Abscissa: time in minutes. Ordinate: resistance on a log. scale. Curves A 1 and B 2 have been displaced on the ordinate for clarity.
A 1: Aqueous humour 8 eyes.
A 2: Aqueous humour, 4 eyes.
B 1: Buffer 4 eyes, the companions to those of A 2.
B 2: Buffer 6 eyes.
H, the isolated point at 200 min. represents the average resistance after injection of 5 v. r. u. Hyalase into the anterior chamber of the eyes of A 1.
All averages are geometrical means. The slopes are directly comparable.

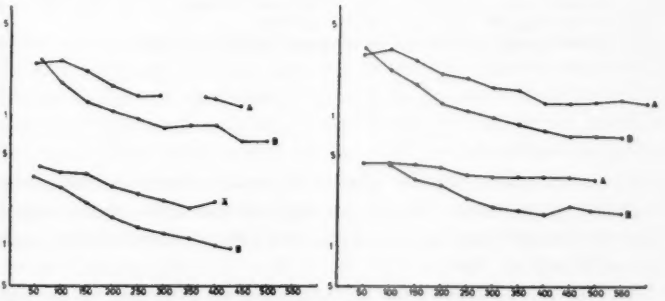

Fig. 15. Perfusion with aqueous and buffer compared in eyes belonging to the same animal.

Abscissa: time in minutes. Ordinate: resistance as in Fig. 2 but on a log. scale. The two upper pairs of curves have been moved upwards one log-unit.
Each pair of curves shows result from one pair of eyes belonging to the same animal. The geometrical mean of these curves is given in Fig. 14.
A: Perfusion fluid aqueous, B: buffer.

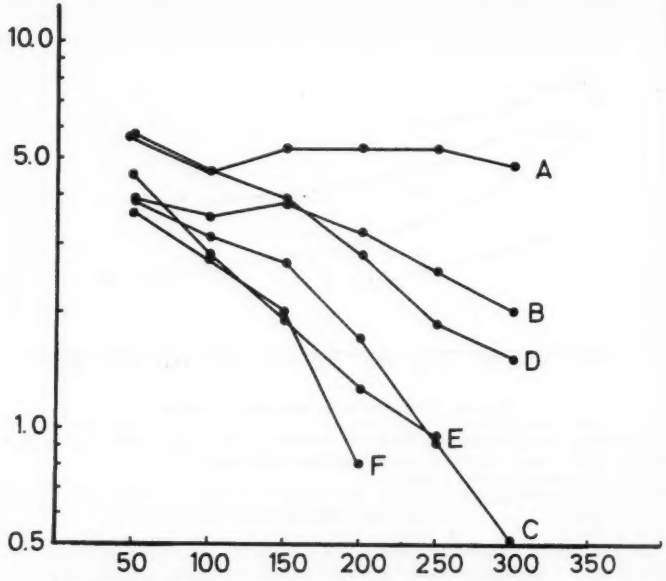

Fig. 16. Effect of pH on the resistance during prolonged perfusion with buffer.

Abscissa: time in minutes. Ordinate: resistance units as in Fig. 2.

The curves have not been vertically displaced against each other. At 100 min., the initial perfusion fluid of pH 7 was replaced by other barbital buffers of equal ionic strength but of different pH. These were allowed to run into the eyes without removing the anterior chamber contents.

A: pH of second buffer 6.76; of chamber contents at end: 6.02
B: » » » » 7.56; » » » » » 7.32
C: » » » » 7.79; » » » » » 7.62
D: » » » » 8.09; » » » » » 7.80
E: » » » » 8.65; » » » » » 8.03
F: » » » » 8.95; » » » » » 8.05

aqueous to which, in the case of 9 eyes, sodium diethyldithiocarbamate was added to give a final concentration of 250 μg/ml. The percentage drop in resistance over 200 minutes was the same for each set.

Fig. 16 shows the effect of perfusion with buffers of various pH values. Because one cannot change the contents of the anterior chamber without eliciting a resistance drop, the pH in the chamber changed continuously during the perfusion. It is clear that alkalinity accelerates the resistance drop normally found with buffer while acidity has the reverse action.

Perfusion of eyes for long periods was also carried out with

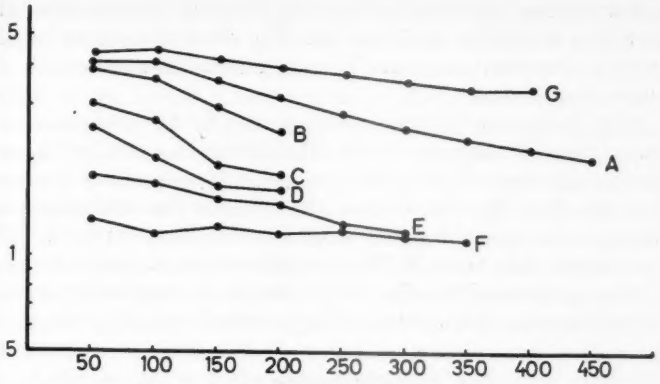

Fig. 17. Perfusion with aqueous or buffer after injection of hyaluronidase.

Abscissa: time in minutes; Ordinate: resistance on a log. scale. The curves are displaced on the ordinate to avoid overlap.

		Initial injection
A.	Aqueous containing 3 v. r. u. Hyalase/ml.	5 v. r. u.
B.	Buffer » 0.5 » »	2 v. r. u.
C.	» » 1.0 » »	4 v. r. u.
D.	» » 0.1 Hyason units/ml	0.2 units
E.	» after washing away initial injection of 5 v. r. u. Hyalase	
F.	Aqueous containing 3.5 v. r. u. Hyalase/ml, towards the end a corresponding amount of Kinaden. Initial injection 33 v. r. u. Hyalase.	
G.	Aqueous after washing away initial injection of 5 v. r. u. Hyalase.	

The number of eyes used for the curves was:
A: 6; B: 3; C: 3; D: 3; E: 5; F: 6; G: 3; the averages are geometric means.

aqueous and with buffer after an initial injection of hyaluronidase. In one series the initial dose was removed while in another the hyaluronidase concentration was more or less maintained. We are therefore concerned here with the behaviour, in the presence or absence of hyaluronidase, of that part of the outflow resistance which was formerly known as hyaluronidase-insensitive.

To remove the initial dose the double syringe arrangement was used. 150 ml of washing fluid were passed through the anterior chamber after 5 v. r. u. of Hyalase had been allowed to act for 30 minutes. In experiments where buffer was to be the perfusion fluid it was also used for the washing while when aqueous was to be the perfusion fluid, washing with 140 ml of buffer was followed by 10 ml of aqueous.

Further experimental details are given in Fig. 17. A relatively low concentration of Hyason was used in view of its potency.

From Fig. 17 it can be seen that perfusion with aqueous induces

a slow decrease in resistance (curve G), and that this is accelerated by 3 v. r. u. Hyalase/ml (curve A). The effect of a higher initial enzyme concentration (curve F) is to reduce the resistance to a stable low level.

With buffer on the other hand (curve E) the breakdown is faster than with aqueous (curve G) although to a smaller degree than in the case of the resistance before hyaluronidase (curves A 2 and B 1, Fig. 14). There is also evidence that hyaluronidase increases the rate of breakdown with buffer. Thus curves B C D are steeper than curve E. The companion eyes to those of curve G were perfused with buffer and yielded an average result almost coinciding with that of the similarly treated eyes of curve E.

Discussion.

Aqueous humour concentrated after dialysis contained no hyaluronic acid-depolymerising activity; no spontaneous decrease in the turbidity producing material of the native aqueous could be detected, and all the depolymerising activity detected by the viscosity measurements had the characteristics of a non-enzymatic process. It can therefore be concluded that a protein with enzymatic properties similar to tissue or bacterial hyaluronidase is absent from the aqueous humour. We have set an upper limit to that concentration of enzyme, the presence of which would be consistent with our findings, and we are unable to support the contention of MEYER (1947) that there is a considerable hyaluronidase activity in cattle aqueous. The experimental results of MEYER have not been published in detail and we are therefore unable to offer any explanation of this divergence.

The weak depolymerising activity present in cattle aqueous could only be detected by using a very highly polymerised substrate. The properties of this depolymerase are very similar to those of the small molecular weight substances which were shown by SKANSE and SUNDBLAD (1943) to degrade hyaluronic acid by oxidation. It appears from the results of these authors that this process requires a substance like ascorbic acid or cysteine, oxygen, and an activating material. Under the conditions of their experiments Cu^{++} was the activating material and similarly, under the conditions of our viscosimetric assay, aqueous only had an activity in the absence of diethyldithiocarbamate.

It is of importance to consider the possibility that oxidative

depolymerisation of hyaluronic acid affects the stability of the angle structures in the perfused eye. The conditions obtaining in the viscosimeter are different from those in the eye. Thus while in the former a heavy metal is the activating agent, this need not be so in the eye where traces of, for instance, H_2O_2 may have a similar effect. We have no information on the oxygen tension in the anterior chamber under the conditions of our perfusions but as the perfusion aqueous was kept under CO_2 and N_2, and the eyes were a few hours old, it is likely to be smaller than in the viscosimeter. The concentration of ascorbic acid, or similar material is not critical. In the light of these considerations it would appear that the only way in which the activity of the aqueous in the perfused eye could be greater than in the viscosimeter would be if the concentration of activating substance were the limiting factor in both cases and higher in the angle structures. It is probable that this is not so, for the activity of perfusion fluid collected from the limbus is smaller than that of the perfusing aqueous. In the absence of evidence in their favour hypothetical activating agents other than heavy metals will not be considered further.

In as much therefore as diethyldithiocarbamate does not inhibit the massage effect, or the slow decrease in resistance to flow of the aqueous on prolonged perfusion it is unlikely that oxidative depolymerisation of the hyaluronic acid in the angle operates in these cases. The magnitude of the activity is consistent with this conclusion for the greatest activity which has been detected in the aqueous is less than the equivalent concentration of Hyalase the presence of which in the aqueous is necessary to induce a just detectable decrease in resistance. It may be noted that this is not rigorous evidence for it involves comparison of activities on different substrates and by different methods of assay.

Evidence has been obtained in the course of this work that the accelerated resistance drop which follows mechanical trauma, the massage effect, may sometimes be analysed into a rapidly occurring and a slowly occurring component. The latter, because of its time course and its temperature dependence has some of the characteristics to be expected for a slow chemical reaction. It is not yet clear whether the first of these phases is a necessary preliminary to the second. However, many of the massage effects which were induced in washed eyes had the former phase without the second. It is possible that the massage effect may, in these

cases at least, be due to an initial mechanical derangement of the angle, making a sensitive component accessible. There may be other ways in which mechanical trauma acts, but it is clear from the present investigation that massage as used here does not liberate a hyaluronidase into, or increase the hyaluronic acid depolymerising activity of the aqueous humour.

We have found that long perfusion with buffer or aqueous humour lowers the resistance but that the latter is better able to preserve the barrier. Here we are in disagreement with WATILLON, PRIJOT and WEEKERS (1954) who have found no drop in resistance on perfusion with aqueous for 300 minutes at which time however, hyaluronidase had lost its effect. We find a resistance drop and also roughly normal sensitivity to hyaluronidase after 280 minutes.

There is no ready explanation for the ability of aqueous humour to keep up the resistance. Buffer and aqueous have a similar pH, molarity and ionic strength. It is improbable that they differ greatly in their reaction with extra-cellular structures. On the other hand, cells occupy a considerable space in the meshwork of the filtering angle (VIRCHOW 1905). Aqueous but not buffer is their normal environment.

From the perfusion of eyes after an initial injection of hyaluronidase, we may conclude that the resistance formerly called hyaluronidase-insensitive decreases on continued perfusion. The resistance decrease is accelerated by hyaluronidase, although high concentrations are needed for an effect to be observed. It thus appears that a substrate for Hyalase enters into the organisation of the angle structures at several levels. At one it is rapidly affected by low concentrations of enzyme, while at another only slowly by very high concentrations. While the first of these actions is most probably due to the hyaluronidase activity this need not be so for the second. MALMGREN (1953) found no proteolytic enzyme, phosphatase, pyrophosphatase, or glucuronidase in Hyalase but the presence of other activities in very high concentrations of the enzyme preparation cannot be excluded.

Summary.

A study has been made of some factors which might be of importance for the hydrodynamic resistance of the angle of the anterior chamber. The possible occurrence in the aqueous humour

of chemical agents affecting the resistance has been investigated and the nature of the barrier studied with the aid of hyaluronidase. The main results are:

1. No hyaluronidase could be detected in cattle aqueous humour.

2. Aqueous humour contains hyaluronic acid capable of being hydrolysed by added hyaluronidase.

3. Aqueous humour has a diffusable thermo-stable component which reduces the viscosity of the hyaluronic acid-protein complex from synovial fluid. The action of this component is inhibited by diethyldithiocarbamate.

4. No increase in hyaluronic acid-depolymerising power is observable in aqueous humour after induction of the gradual breakdown of resistance caused by mechanical trauma.

5. Mechanical trauma sometimes seems to act on the angle through an immediate physical derangement followed by a slower, temperature-dependent phase. This latter is not inhibited by diethyldithiocarbamate.

6. The fast initial resistance drop caused by small amounts of testicular hyaluronidase can also be induced by staphylococcal hyaluronidase.

7. On perfusing the angle for 8 hours with buffer, the resistance decreases continuously. With aqueous humour as perfusate the resistance is stable for about 100 minutes and then decreases. The hyaluronidase-sensitive component in the angle does not disappear for several hundred minutes when perfused with aqueous.

8. In addition to its immediate action in low concentrations, hyaluronidase in high concentrations induces a further slow decrease in resistance of the angle.

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